THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on May 3rd, the President, Professor W. H. Roberts, in the chair.

The following were elected members of the Society:—J. B. Firth, D.Sc., M.I.Chem.E., F.I.C.; G. Isles; C. H. Wordsworth, B.Sc., A.I.C.

Certificates were read in favour of the following candidates for membership:—D. O. Burgess; V. C. Marquez, D.Sc.; J. Kay; C. A. Macdonald, B.Sc., F.I.C.; A. Srinivasan, M.A.

The following papers were presented and discussed:—"Extract of Malt with Cod Liver Oil: Determination of Oil and Vitamin A," by D. C. Garratt, B.Sc., Ph.D., F.I.C.; "The Presence of Leuco-anthocyanins in Criollo Cacao," by the late A. W. Knapp, M.Sc., M.I.Chem.E., F.I.C., and J. F. Hearne, A.I.C.; "Notes on the Examination of Textiles in Cases of Suspected Dermatitis," by H. E. Cox, Ph.D., D.Sc., F.I.C.

SCOTTISH SECTION

An Ordinary Meeting of the Scottish Section was held in the North British Station Hotel, Edinburgh, on April 28th. Mr. T. Cockburn, the Chairman, presided over a large attendance of members and friends.

The following papers were read and discussed:—"Counterfeit Coins," by A. Scott-Dodd, B.Sc., Ph.D., F.I.C.; "The Assay of Mercury," by James Sandilands, F.I.C.; "Description of a Closed Respiration Apparatus for Plant Tissue," by A. M. Smith, Ph.D., D.Sc., A.I.C.

Death

We deeply regret to record the death of Kenneth Edward Nethercoate Williams, a member of the Society since 1923.

Obituary

RICHARD AUGUSTUS CRIPPS

RICHARD AUGUSTUS CRIPPS died after an operation in a nursing home at Hove on March 31st, 1939, at the age of 76.

Cripps was born at Goring, Sussex, and served his apprenticeship with Mr. J. H. Read, pharmaceutical chemist, Upper Holloway, London. In 1881 he was Jacob Bell Memorial Scholar of the Pharmaceutical Society, and in the following year was awarded that Society's bronze medal for practical chemistry. For two years he acted as demonstrator for Professor Attfield, and later became an assistant in the laboratory of Messrs. P. W. Squire.

In 1884 he passed the "Major" examination of the Pharmaceutical Society, and then became analyst to Messrs. Southall Bros. and Barclay, Birmingham. He was elected a Fellow of the Institute of Chemistry in 1888, and in 1893 set up in private practice as an analyst at Haywards Heath, Sussex. In addition to his consulting work he established, in 1895, a business as wholesale manufacturer of galenicals and concentrated infusions. This met with such success that in 1904 he opened extensive premises at Hove, and, in association with his son, Mr. R. D. Cripps, was able to extend its scope and ultimately to convert it into a private company.

Cripps was elected a member of our Society in 1896, and served on the Council in 1910–11. In 1901 he was appointed Public Analyst for the County Borough of Bournemouth, a post that he held for thirty-two years. His official work, however, occupied only part of his time, and he was able to do some original work. Between 1883 and 1928 he contributed fourteen papers to the *Pharmaceutical Journal*. To THE ANALYST he made four contributions: "Notes on the influence of Boric Acid upon the Action of the Digestive Ferments" (1897); "Examination of Cough Mixture" (1902); "The Determination of Essential Oil and Moisture in Spices and Aromatic Drugs" (1909); "The Presence of Sulphates in Flour" (1914).

The papers that he prepared for the British Pharmaceutical Conference included: "An Examination of some Commercial Concentrated Infusions" (1905); "Decoctum Aloes Co. Concentratum; Notes on the Research upon Ipecacuanha" (1895); "Notes on Unguentum Cocainae B.P." (1906).

He was the author of a book, *Galenic Pharmacy*, popular with students of pharmacy thirty or forty years ago. As founder of the Cripps Exhibition (value £21), tenable at The Brighton Technical College, he acted as examiner in conjunction with other pharmacists; also, at various times he read papers to the evening students on subjects so wide apart as: "Coal Tar Distillation and some of its Products," "Note on the New Pharmacopoeia, Tincture Deposits," and the "Estimation of the Alkaloids of Conium."

Such a wealth of work from one man speaks volumes for his energy. Cripps belonged, however, to the old school who, after a hard day's toil, thought nothing of staying late in order to clear up points that the work of the day had left obscure. For such men we, a younger generation, have much to be thankful.

My first acquaintance with him was through his great friend, the late Mr. E. H. Farr, pharmacist, of Uckfield, who at that time was examiner for the

Pharmaceutical Society. Later, Cripps and I often met, generally in opposition, both in and out of Court, but we always remained good friends whether the result was a win, a loss or a draw.

Cripps was a man of deep religious feeling. At his funeral, at which I represented our Society, no one could fail to be struck by the very large number of pharmacists present to pay their last tribute of respect. The impressive words of the officiating clergyman were an eloquent testimony not only to the high regard in which Cripps was held by his professional colleagues, but also to the affection felt for him by the members of the church he had so regularly attended, and in which he had taken such a practical interest. S. ALLINSON WOODHEAD

The Determination of Traces of Zinc in Biological Material and Natural Waters

BY NOEL L. ALLPORT, F.I.C., AND C. D. B. MOON, A.I.C.

(*Read at the Meeting, April* 5, 1939)

RECENTLY, considerable interest has attached to the determination of the minute traces of zinc now known to be widely distributed in plant and animal tissues and possibly playing a part in the biological economy. In the absence of suitable specific colour reactions it is necessary first to separate the zinc, and various proposals have been made to effect this by extraction with a solution of dithizone (diphenyl-thiocarbazone) in chloroform or carbon tetrachloride.[1,2,3] In our hands these methods did not prove to be satisfactory. Sylvester and Hughes[4] recommend extraction with a chloroform solution of dithizone from an aqueous medium buffered with ammonium acetate at about *p*H 4·5, and in the early stages of this work these conditions led to promising results. Later, when the determination of the isolated zinc had been perfected, this method of extraction was found to be unreliable, particularly for minute traces when relatively large proportions of heavy metals and iron were also present. Further, the method is inconvenient for the examination of natural waters without first resorting to preliminary evaporation of large volumes of the sample, which involves the risk of inaccuracies due to contamination by zinc from the vessels used.

Some years ago del Campo Cerdan and Puente[5] found that when resorcinol is added to an alkaline solution of a zinc salt and the mixture is protected from oxidation, a purple colour is produced. Although too limited in application to be satisfactory as a colour test, this reaction suggested that the presence of resorcinol might facilitate the extraction of traces of zinc from an alkaline medium by means of a chloroform solution of dithizone. Repeated trials have shown this supposition to be justified.

In the method here described, the zinc, together with any heavy metals, is extracted with dithizone from an alkaline tartrate solution containing resorcinol, and the metals are transferred from the chloroform solution of dithizone by

shaking with $N/10$ hydrochloric acid. The heavy metals are removed by means of hydrogen sulphide, the filtrate is evaporated, and the zinc is precipitated with quinaldinic acid (α-quinoline carboxylic acid) according to the method of Rây and Bose.[6] The precipitate of zinc quinaldinate is exceptionally granular and easy to manipulate. If a microchemical balance is available the determination may be completed gravimetrically; alternatively, the colorimetric method which we describe has proved to be quite convenient for quantities of zinc ranging from 5 to 50γ. The various suggestions for the colorimetric or titrimetric determination of minute quantities of zinc with dithizone[3,7,8,9,10,11,12] either failed to give reliable results or were inapplicable to this particular work. Loss of zinc by adsorption during the removal of heavy metals as sulphides[13,14] was prevented by precipitating from a mixture composed of equal volumes of $N/10$ hydrochloric acid and acetone.

METHOD.—*Reagents.*—Water and all chemicals should be of analytical reagent quality. The solution of dithizone in chloroform must be specially purified not more than two days before use in the following manner:—Dissolve 0·1 g. of dithizone (diphenylthiocarbazone) in 100 ml. of chloroform (analytical reagent quality) and extract with three 25-ml. portions of 5 N hydrochloric acid. Reject the aqueous layers, add 100 ml. of water and 5 ml. of ammonia (sp.gr. 0·880), shake for one minute, allow the layers to separate, reject the chloroform layer and wash the aqueous layer with three further separate portions of chloroform. Add 100 ml. of chloroform, acidify the aqueous liquid with hydrochloric acid, shake vigorously, allow the layers to separate, and draw off the chloroform solution and wash it repeatedly with water until the washings are neutral in reaction; this washed chloroform solution constitutes the purified dithizone reagent and should be kept in a stoppered amber bottle.

The sodium quinaldinate solution is prepared by adding 0·2 g. of quinaldinic acid to 3 ml. of water containing 1 drop of phenolphthalein indicator solution, neutralising with $N/10$ sodium hydroxide solution, and finally diluting to 10 ml. with water. This reagent will remain in good condition for two days if stored in a stoppered bottle.

Resorcinol is employed as a 10 per cent. w/v solution in ethyl alcohol (95 per cent.).

Glacial acetic acid, as used in the actual colour test, should contain not less than 99·5 per cent. of hydrogen acetate.

Procedure.—The preliminary treatment will vary according to the nature of the material to be tested. The procedure is described below in its most comprehensive form, applicable to the examination of organic material containing both zinc and a trace of heavy metals:

Incinerate a suitable quantity of the sample to be tested, containing preferably between 20 and 100γ of zinc, in a silica dish, using a muffle furnace at 500 to 550° C., and extract the carbon-free ash by boiling with a little 10 N hydrochloric acid, and then diluting with water and boiling again. Allow the liquid to cool, dilute to about 100 ml., or other convenient volume, add 0·2 g. of tartaric acid for each 100 ml. of liquid; when this has dissolved, transfer the solution to a separating funnel, add 1 ml. of a 10 per cent. alcoholic solution of resorcinol for

each 100 ml. and a few drops of thymol blue indicator solution and then sufficient 5 *N* ammonia to render the colour of the mixture slate blue (approximately *p*H 9). Shake with three successive 10-ml. portions of the purified chloroform solution of dithizone, and transfer the chloroform layers to a second separating funnel; if the third dithizone extract is red or purple, continue the extraction with further portions of the reagent until the chloroform layer remains green. Wash the combined extracts once with 10 ml. of water, run the chloroform solution into a third separating funnel, and shake with four separate 10-ml. portions of *N*/10 hydrochloric acid. Combine the acid layers and wash them free from any mechanically suspended dithizone reagent by pouring small quantities of chloroform through the aqueous liquid without shaking; reject the chloroform used for washing and dilute the acid extract to a convenient known volume with *N*/10 hydrochloric acid. Test a portion of this solution for heavy metals with hydrogen sulphide; if no reaction is obtained, transfer an aliquot part of the solution to a beaker, omit the separation of the heavy metals, and proceed to the determination of zinc, beginning with the evaporation of the acid solution to dryness.

If the slightest trace of heavy metal is present, add to an aliquot part of the solution 1 ml. of a 1 per cent. aqueous solution of cupric chloride for each 50 ml. of solution taken. Boil to remove dissolved chloroform, allow the liquid to cool, add an equal volume of acetone, heat to boiling, and pass in hydrogen sulphide gas until the precipitated sulphides have coagulated. Filter the liquid slowly with the aid of suction through a Gooch crucible previously packed with paper pulp,* and wash with hydrogen sulphide water. Heavy metals having thus been removed, evaporate the filtrate (or if heavy metals were not present originally, evaporate an aliquot part of the original *N*/10 hydrochloric acid extract) to dryness in a beaker of Pyrex glass by gently boiling it over wire gauze. Add to the residue 0·5 ml. of nitric acid and about 5 mg. of potassium chlorate, evaporate to dryness, moisten the residue with a little nitric acid a second time, again evaporate to dryness, and then add a few drops of 5 *N* ammonia and repeat the evaporation. Add to the residue 1 ml. of *N*/2 acetic acid and immerse the beaker in a water-bath adjusted to 60° C. When the residue has dissolved, add 0·5 ml. of sodium quinaldinate solution, *drop by drop*, and continue to warm the mixture at 60° C. for 5 minutes†; then completely transfer the liquid, together with the precipitate of zinc quinaldinate, to a centrifuge tube of 15 ml. capacity, using warm water. Centrifuge, reject the supernatant liquor, wash the precipitate with acetone, and decant the solvent from the precipitate after centrifuging; repeat the washing with acetone twice, and then dry the residue in an oven adjusted to 100° C.

* *Preparation of Gooch Crucible.*—About 0·2 g. of Whatman No. 1, or similar filter-paper is covered with 10 *N* hydrochloric acid and rubbed with a glass rod until thoroughly disintegrated. After dilution with water, the mixture is poured into a Gooch crucible having a base of about 2 cm. in diameter, and the pulp is gently but firmly packed by pressing it with the flattened end of a glass rod and washed with water until free from acid, suction being used.

† If it is preferred to complete the determination gravimetrically, the supernatant liquid should be drawn off by suction through a filter-stick, the precipitate washed with several portions of hot water, and the beaker and filter-stick dried for about 10 minutes at 125° C. in a current of air in a Benedetti-Pichler drier and finally weighed. The compound has the composition $(C_{10}H_6NO_2)_2Zn.H_2O$ and contains 15·29 per cent. of zinc.

Introduce into a dry Pyrex round-bottomed flask of 50 ml. capacity, fitted by means of a ground-glass joint with an air-condenser, 1 g. of phthalic anhydride, 1·5 g. of naphthalene (recryst.) and 0·5 g. of zinc filings (analytical reagent quality). Introduce 2 ml. of glacial acetic acid into the centrifuge tube, gently boil until the zinc quinaldinate has dissolved, transfer the solution with the aid of a thin glass rod to the Pyrex flask, and wash the tube with three separate 1-ml. portions of glacial acetic acid. Attach the condenser to the flask and gently boil the contents for exactly 20 minutes. Decant the partly cooled solution into a 25-ml. stoppered measuring cylinder, wash the flask several times with a mixed solvent consisting of equal volumes of acetone and ethyl alcohol (95 per cent.), add the washings to the contents of the cylinder and, after cooling, adjust the volume of the liquid to 25 ml. with the same mixed solvent. Transfer a portion of this clear yellow liquid to a 2-inch all-glass cell, and measure the intensity of the colour by means of a Lovibond tintometer. Considering only the yellow component, read the amount of zinc from a curve based on the relationship shown below. Make a control determination to correct for minute traces of zinc present as impurity in the reagents employed, refer the yellow component of the colour obtained to the amount of zinc as shown on the same curve, and deduct the result from that obtained in the primary determination.

Zinc present in the actual test	Colour of liquids observed in 2-inch cell Lovibond units		
	Yellow	Red	Brightness
γ			
nil	nil	—	—
10	0·9	0·1	—
20	1·8	0·2	—
30	2·7	0·4	0·1
40	3·5	0·6	0·1
50	4·3	0·8	0·1
60	4·9	0·9	0·1
70	5·4	1·0	0·1

If the yellow component of the colour due to the sample is more than 5·4 Lovibond units when measured in a 2-inch cell, repeat the precipitation and colour test, employing a smaller aliquot part of the reserved portion of the hydrochloric acid extract.

Zinc in Natural Waters.—Acidify a suitable quantity (250 or 500 ml.) with hydrochloric acid, using 1 ml. of 10 *N* acid for each 100 ml. of water, boil for 5 minutes, allow to cool, and add 0·2 g. of tartaric acid and 1 ml. of a 10 per cent. alcoholic solution of resorcinol for each 100 ml. of sample taken. When solution is complete transfer to a separating funnel, adjust to *p*H 9 with 5 *N* ammonia and proceed as described above, beginning with the extraction of the zinc with dithizone.

NOTES ON THE METHOD.—The naphthalene used in the colour test acts as a solvent and prevents the excess of phthalic anhydride from crystallising out of solution as the mixture cools. A large proportion of this reagent was found to be essential to obtain full production of the colour.

In calculating the results from the colorimetric observations, only the yellow component is considered, but, as a guide to accurate matching, the data quoted above include the proportion of red tint and brightness, which was found necessary to effect colour agreement.

Of the more commonly occurring metals, only nickel and cobalt interfere. A relatively large quantity of manganese, even if not removed, has no influence on the precipitation of zinc by quinaldinic acid. No interference has been experienced through the presence of phosphates or any other commonly occurring anions.

RESULTS.—The efficacy of the method was first tested by adding known amounts of standard zinc sulphate solution to 50 ml. of water (analytical reagent quality) and submitting the solutions to the complete process of extraction, precipitation and colour test; the results obtained are shown in Table I.

TABLE I

Zinc added, γ ..	70, 85, 35, 50, 75, 10
Zinc found, γ ..	65, 83, 31, 52, 74, 11

Further experiments were made in which small quantities of zinc were recovered from mixtures containing relatively large proportions of heavy metals, iron, phosphates and other salts. In each instance the materials were initially dissolved in about 50 ml. of water (analytical reagent quality). The results are given in Table II. In all these experiments the colours were matched by independent observers having no knowledge of the anticipated values.

TABLE II

Lead added γ	Iron added γ	Other substances added		Zinc added γ	Zinc found γ
—	500			60	54
—	—	Copper, 500γ		45	46
20	1000			40	31
—	1000			35	36
1000	1000			70	65
200	200	Sodium chloride,	0·5 g.	50	44
		Potassium chloride,	0·1 g.		
		Magnesium sulphate,	0·1 g.		
—	500			50	52
500	500	Mercury, 200γ		80	88
500	500			40	49
—	—	Manganese, 30γ		30	30
—	—	Manganese, 300γ		30	28
500	500			25	25
200	—	Sodium chloride,	0·5 g.	80	81
		Potassium chloride,	0·1 g.		
		Magnesium sulphate,	0·1 g.		
—	—	Sodium phosphate,	1 g.	40	35

The results of a few determinations of the zinc-contents, mainly of foodstuffs and fresh glands, are collected in Table III. The sample of tomatoes was derived from fruit grown under observation in a private greenhouse, and the risk of accidental contamination with zinc from galvanised iron wire, or other source, was eliminated. Of particular interest is the confirmation of an observation already

recorded by Sylvester and Hughes[4] that, whereas a sample of egg-yolk was found to contain 26 parts per million of zinc, none was detected in the white. The sea-water was collected in bottles lined with paraffin wax, risk of the sample becoming contaminated with zinc derived from the glass being thus eliminated.

TABLE III

Product	Zinc found p.p.m.
Red Currants	1·2
English tomatoes	4·3
New-laid egg (yolk)	26·0
New-laid egg (white)	nil
Pancreas	13·6
Thyroid gland	10·9
Anterior pituitary gland	20·6
Ditto	29·7
Posterior pituitary gland	12·5
Tap water from London mains	0·23
Sea water collected off N. coast of Cornwall	0·06

SUMMARY.—The extraction of traces of zinc from aqueous liquids with a chloroform solution of dithizone has been investigated, and the process has been made quantitative by the use of resorcinol.

Conditions for separating traces of zinc from relatively large proportions of heavy metals have been studied, and a method for effecting the removal of the latter as sulphides without loss of zinc by adsorption is suggested.

A colorimetric test for the determination of the isolated zinc is proposed.

The method provides a means for accurately determining minute traces of zinc present in biological tissues, foodstuffs and natural waters.

The sensitivity of the colour test is such that determinations of zinc can be conducted on small samples, while in the examination of natural waters the preliminary evaporation of large volumes is obviated.

Nickel and cobalt are the only commonly occurring metals which interfere with the proposed method for determining zinc.

We are indebted to the Directors of The British Drug Houses, Ltd., for permission to publish the results of this investigation.

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Discussion

Mr. D. D. Moir asked if the final reaction liquid, after having been refluxed in the presence of metallic zinc, could be easily separated from the metal in a condition suitable for observation of its colour intensity.

Mr. A. L. Bacharach asked if the authors were satisfied that in their process nothing but zinc could be precipitated by the quinaldinic acid.

Mr. J. R. Nicholls wished to know if any trouble had been encountered as a result of zinc being extracted from glass-ware.

Dr. B. S. Evans also remarked that he had often encountered difficulties arising from zinc present in glass-ware; this was very liable to be extracted by boiling alkaline solutions. Had the authors had any experience with the separation of zinc from cadmium? He, himself, had not been able to separate small quantities of zinc from large quantities of cadmium; in his experience attempts to remove the latter as sulphide resulted in heavy loss of zinc by adsorption.

Mr. Moon, replying, explained that the zinc used to promote the colour reaction was in the form of filings and that the supernatant liquid could be readily poured off in a perfectly clear condition. As to the possibility of other metals besides zinc being precipitated by the quinaldinic acid, nickel and cobalt were the only ones of common occurrence which could interfere, since the method provided for the removal of all others which formed insoluble quinaldinates. As regards zinc contamination by glass-ware, trouble had been experienced, but Pyrex glass appeared to be satisfactory. In the method described, the boiling of alkaline liquids in glass vessels was avoided. The authors had not paid any special attention to the question of separating the zinc from relatively large quantities of cadmium, as it was not anticipated that this problem would arise in the examination of biological tissues or natural waters.

Dr. J. H. Hamence remarked that he was sorry to hear that the authors had not found the oxine method suitable for their purpose; he rather favoured that reagent for the determination of zinc when present in traces. Had the authors ever found discrepancies in the Lovibond readings made by different observers?

Mr. R. W. Sutton said that in his laboratories they had studied the application of the nephelometer to the determination of traces of zinc by means of the ferrocyanide reaction, and had found that they could get accurate results provided that every operation was carefully standardised. It seemed that this might be a suitable method for determining zinc in cadmium. He was surprised to find the authors advocating the removal of other metals by means of hydrogen sulphide; he, himself, had never been satisfied that this time-honoured procedure really did provide a satisfactory separation for zinc but, presumably, the authors' use of acetone overcame adsorption difficulties. He recalled a paper published in Germany about two years ago which prescribed conditions for the successive determination of copper, lead and zinc; in this method the first metal was extracted with dithizone from an acid aqueous solution of the sample, the lead was then removed after neutralisation of the filtrate and addition of potassium cyanide and, finally, the zinc was extracted from the same aqueous liquid after it had been boiled with excess of mineral acid to remove cyanide and the reaction adjusted with sodium acetate.

Mr. Allport, in reply, emphasised the point that quinaldinic acid was an exceptionally good precipitating reagent for zinc and, in this respect, was better than oxine; hence it was possible to deal with very small quantities of zinc (for example, 5 to 10μ) without risk of loss. As regards colour measurement by means of the Lovibond tintometer much depended upon the correct adjustment of the brightness and the limitation of the tints to low denominations. Values for brightness, as well as for the red component observed to accompany the yellow tints, were included in the paper as guides to correct colour matching. In all experiments the colours were matched by independent observers having no knowledge

of the anticipated results, and in this way the risk of personal bias was eliminated. With regard to the ferrocyanide method the necessity for making a series of standards for every determination constituted a serious disadvantage, even presuming the actual turbidities could be accurately observed. The German method to which Mr. Sutton alluded was that of Strohecker, Riffart and Haberstock (*Z. Unters. Lebensm.*, 1937, **74**, 155) and was cited in the present paper. The method had been tried, but the results obtained were not promising. Summarising, Mr. Allport observed that the method they had proposed had been proved suitable for work within the limits defined by the title of the paper, but he could not vouch for its utility in any wider application, as this had not been investigated.

A Rapid Method for the Determination of Traces of Bismuth in Lead

By R. G. ROBINSON, B.Sc., A.I.C.

The sharp separation of very small amounts (about 0·001 per cent.) of bismuth in lead is a matter of some difficulty, mainly because lead sulphate persistently retains bismuth.

The ingenious method of internal electrolysis used by Collin[1] would appear to be applicable with difficulty to concentrations of this order.

B. S. Evans,[2] by separating the maximum amount of lead as chloride, minimises the retention of bismuth, but it is the writer's experience that this is by no means negligible when a sulphuric acid solution, containing lead, even in small amount, is heated to the fuming point. As an illustration of this, 3 ml. of a solution containing 0·30 mg. of bismuth as sulphate, 2 ml. of a solution containing 20 mg. of lead as nitrate, and 20 ml. of dilute (1 : 1) sulphuric acid were mixed and heated on the hot plate until the acid fumed, a control experiment without lead being made simultaneously. As soon as fuming of the acid occurred, the beakers were allowed to cool, and 60 ml. of water were added to each. The solutions were gently boiled for 5 minutes, cooled and filtered, and the bismuth was determined by the colorimetric method to be described below. Comparison of the two experiments showed that the lead present in the first had caused a loss of 0·10 mg. of bismuth.

In devising the following method it was desired to avoid this possible source of error, and also to reduce the time by dispensing with any process of evaporation. The scheme finally adopted comprises the following features:

(1) Solution of a 20-g. sample in dilute nitric acid, followed by separation of the major part of the lead as chloride. (2) Co-precipitation of the bismuth with a small amount of ferric iron precipitated as hydroxide. (3) Colorimetric determination of the bismuth as iodide by use of a Lovibond Tintometer. The colour can be measured by means of the yellow units alone.

Attention was first given to the colorimetric determination proper. The presence of ferric iron necessitated the use of an effective reducing agent, but sulphur dioxide, commonly used for this purpose, has serious disadvantages. A moderate excess produces a yellow colour which has been stated to be due to iodine

(*cf.* Kruisheer[3]). This assumption seems to be ill-founded, since the yellow substance does not react with starch, and it may easily be confused with bismuth iodide, with which it is identical in colour. After many trials, hypophosphorous acid was found to be a suitable reagent, and it is interesting to note that Fitter,[4] whose priority must be acknowledged, agrees with Nickolls[5] as to the inconvenience of sulphur dioxide and uses hypophosphites in a method for the determination of bismuth in copper.

Hypophosphorous acid is conveniently obtained as a solution (sp.gr. 1·135), and it is to this solution that subsequent reference is made. The reducing action depends largely on the acidity and temperature of the solution, but, in all the trials made, it was found sufficient to warm the liquid to about 70° C. to effect rapid reduction of iodine.

It was found that the bismuth iodide colour reaction was moderately tolerant to variation in acidity, temperature, and iodide concentration, and after consideration of the effect of antimony on the determination, the following conditions were adopted as standard.

The solution, containing 5 ml. of (1 : 1) sulphuric acid, 5 ml. of hypophosphorous acid, and 3 g. of tartaric acid in 100 ml., was poured on to 1·5 g. of solid potassium iodide. After the iodide had dissolved the solution was warmed to 70° C. and then cooled to 20° C., and the colour was measured in daylight in a 1-in. cell. The calibration values given below were obtained with bismuth added as a dilute sulphuric acid solution containing 0·0001 g. of bismuth per ml.

Bismuth added, mg. ..	0·10	0·20	0·25	0·30	0·35	0·40
Yellow units (Lovibond) ..	0·7	1·4	1·7	2·1	2·5	2·9

These values show that the relationship is linear within the limits of error of this method of determining colour, 1 unit of yellow representing 0·143 mg. of bismuth. In using the tintometer it was noticed that yellow is not the easiest of the primary colours to match, and in consequence it was important to avoid optical fatigue. Whenever possible, estimations were checked by another observer without knowledge of the amount of bismuth present, and these values seldom deviated by more than 0·1 unit yellow. The figures given above are taken from a large number of observations which, for the sake of brevity, are not quoted. As noted by Fitter, the colour is very stable, and solutions were exposed to the laboratory atmosphere for 24 hours without change in colour.

As regards the conditions for the colorimetric assay, it was found that, to avoid interference by antimony, it was important to have not more than 5 ml. of dilute (1 : 1) sulphuric acid. If, for example, 20 ml. of the dilute acid were used, and the sample to be assayed contained about 0·25 per cent. of antimony, an indefinite increase in colour of up to 10 units yellow over that expected from the known bismuth-content was observed. This colour was proved to be due to antimony. It faded completely on dilution, and also if the solution was heated to the boiling-point, although it partly returned on cooling. Experiments in which antimony alone was present showed that if only 5 ml. of dilute (1 : 1) sulphuric acid were present, no colour was formed, and it was subsequently proved that under these conditions 5 mg. of antimony caused no interference with the determination of bismuth. When the sample contains an appreciable amount of

antimony, tartaric acid is necessary if perfect extraction of bismuth from the ferric hydroxide precipitate is to be effected.

The separation of bismuth together with ferric hydroxide was investigated in tests on a series of solutions containing 0·3 mg. of bismuth, 5 mg. of ferric iron, and 100 mg. of lead, in a mixture of 30 ml. of hydrochloric acid and 130 ml. of water. When iron was precipitated with ammonia in the normal manner, and the precipitate was extracted with a warm sulphuric and tartaric acid solution, approximately only two-thirds of the bismuth present was obtained, the ferric hydroxide precipitate contained much lead, and the filtrate deposited lead hydroxide on cooling. When, however, the solution was free from lead, the bismuth added was quantitatively recovered. Attempts to improve the recovery in presence of lead invariably failed if ammonia was used, but after many trials it was found that a successful separation could be obtained by neutralising carefully to a given point with sodium hydroxide, and, further, that the necessary conditions were rendered less critical by use of an ammonium acetate buffer. Ferric hydroxide thus precipitated carried down only small amounts of lead, and there was no precipitation of lead hydroxide if the solution was cooled. It was not found necessary to digest the precipitate, and in practice it was usually filtered off immediately after precipitation. The maximum amount of bismuth tested was 0·4 mg., and 5 mg. of ferric iron proved sufficient to collect this amount. Antimony, present to the extent of 50 mg., had no ill-effect on the recovery of the bismuth.

THE COMPLETE METHOD.—The lead sample was rolled to a thin tape, and 20 g. were weighed into a 400-ml. beaker and treated with 50 ml. of water and 25 ml. of nitric acid. The beaker was covered and placed on the water-bath until the sample had dissolved. Hydrochloric acid (30 ml.) was then added to precipitate the lead, the contents of the beaker were mixed, cooled to 20° C. or lower, and filtered with the aid of suction through a plug of cotton-wool into a small Buchner flask, and the beaker and precipitate were washed three times with cold (1 : 1) hydrochloric acid. The filtrate was returned to the beaker, which had been washed free from adhering lead chloride, and 5 ml. of a solution containing 1 mg. of ferric iron per ml., followed by 20 ml. of 20 per cent. ammonium acetate solution were added. Sodium hydroxide pellets were then gradually added, while the solution was stirred, until a small amount of lead hydroxide was precipitated. (*Note.*—As progressive additions of sodium hydroxide are made to such a solution, ferric hydroxide is precipitated while the solution is still faintly acid. Further addition of the alkali causes precipitation of lead hydroxide, which redissolves when the solution becomes strongly alkaline, leaving the ferric hydroxide in an apparently uncontaminated condition. Unfortunately, in all tests in which an attempt was made to use this ferric hydroxide precipitate, the bismuth recovery was poor and variable, being at best equal to two-thirds of the amount added; on the other hand, with the ferric hydroxide formed when the solution was sufficiently acid to prevent a visible precipitate of lead hydroxide there was complete recovery of bismuth in every instance.)

After addition of sodium hydroxide as described, the solution was titrated with strong hydrochloric acid, added dropwise with efficient stirring, until the visible lead hydroxide precipitate had just dissolved. A further addition

of 1 ml. of acid was made, the ferric hydroxide precipitate was filtered off immediately on a small asbestos mat with the aid of suction, and the beaker and precipitate were washed three times with cold water, the filtrate being rejected. Tartaric acid (3 g.) was dissolved in the beaker in a mixture of 45 ml. of water with 5 ml. of (1 : 1) sulphuric acid, the solution was warmed to about 80° C. and poured on to the precipitate, and the filtrate was collected in a 250-ml. flask, being allowed to flow through the filter by gravity alone, since if suction were applied too early, a faint precipitate of lead sulphate appeared in the flask. When the extracting solution had passed through, the filter was washed twice with water, with the aid of suction, and discarded. The filtrate was treated with 5 ml. of hypophosphorous acid, diluted to 100 ml. with water, and poured into a dry flask containing 1·5 g. of potassium iodide. The liquid was then heated to about 70° C. and cooled to 20° C., and the colour was measured in daylight in a 1-in. cell.

A series of tests of the method is given in Table I, the materials taken for the determinations being:

(A) 20 g. of bismuth-free lead plus 0·1 g. of lead alloy containing 0·1 per cent. of bismuth.

(B) ,, ,, ,, ,, ,, 0·2 g. ,, ,, ,, ,, ,, ,, ,, ,,

(C) ,, ,, ,, ,, ,, 0·3 g. ,, ,, ,, ,, ,, ,, ,, ,,

(D) ,, ,, ,, ,, ,, 0·4 g. ,, ,, ,, ,, ,, ,, ,, ,,

(E) 20 g. of a works batch sample, containing 0·25 per cent. of Sb, 0·01 per cent. of As, 0·10 per cent. of Cu, and 0·25 per cent. of Ag as the major impurities.

(F) 20 g. of a sample of the same batch after softening and desilverising, the impurities now being 0·01 per cent. of Sb and 0·51 per cent. of Zn.

(G) 20 g. of the same batch at a further stage in the refining process, the impurities being 0·01 per cent. of Sb and 0·01 per cent. of Zn.

(H) 20 g. of the same batch after final refining.

In connection with samples (E) to (H), it is the experience of the lead-refining industry that the refining processes carried out as in this instance do not affect the bismuth-content of the lead.

TABLE I

	Bismuth added Per Cent.	Units, yellow	Bismuth found Per Cent.
(A)	0·0005	0·7	0·0005
(B)	0·0010	1·5	0·0011
(C)	0·0015	2·0	0·0014
(D)	0·0020	2·8	0·0020
(E)	nil	2·1	0·0015
(F)	nil	2·1	0·0015
(G)	nil	2·1	0·0015
(H)	nil	2·1	0·0015

With sample (E), which contained 0·25 per cent. of antimony, some care was needed to recognise the correct point when adjusting the solution for precipitation, but the relatively high copper-content did not interfere in any way with the analysis.

Bismuth-free lead was prepared by partially scorifying lead containing 0·0015 per cent. of bismuth, and reducing the resulting litharge by fusion with sodium cyanide. The reduced metal showed complete absence of bismuth within the limits of the method.

Lead-bismuth alloy containing 0·1 per cent. of bismuth was made from bismuth purchased as pure, diluted in two stages with lead containing 0·0015 per cent. of bismuth.

The standard bismuth solutions used for calibration were prepared from the same sample of pure bismuth, and also from bismuth oxide. Weak sulphuric acid solutions, containing 0·0001 per cent. of bismuth, were simply prepared by dissolving bismuth or bismuth oxide in hot strong sulphuric acid and diluting; these solutions proved quite stable. Unless otherwise stated, all acids mentioned are to be taken as being the usual concentrated acids of analytical reagent quality.

The method has been in use for eighteen months in connection with refined lead. If it is required to determine bismuth in antimonial lead, it is necessary to introduce a preliminary separation, which, incidentally, is useful in connection with the determination of various other impurities. It is hoped to describe this separation at a later date.

My acknowledgments are due to the management of Britannia Lead Co., Ltd., Northfleet, Kent, for permission to publish this paper.

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BRITANNIA LEAD CO., LTD.
NORTHFLEET
KENT

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The Action of Light on Wool with particular reference to the Production of Acid. I

BY S. R. TROTMAN, M.A., F.I.C., AND W. W. TAYLOR, B.SC., F.I.C.

THE action of sunlight and ultra-violet light on wool may be considered from two points of view, *viz.* (1) destruction of wearing properties and alteration of affinity for dyestuffs; (2) the chemical changes that take place.

The first of these, commonly termed "weathering," is due to a combination of agencies including bacterial action[1] and traces of atmospheric ozone.[2] Whittaker[3] appears to have been the first to investigate the chemical changes that occur, and said that weathering is due to de-amination. Fort and Lloyd,[4] on the other hand, found that exposure to light causes an increase in amino groups, and Krais[5] held that neither sunlight nor ultra-violet light has any action on wool. Bergen[6] was the first to observe that exposure of wool to ultra-violet light causes the production

of acids, owing to part of the sulphur being oxidised to sulphuric acid. Meunier and Rey[7] found that exposure to light causes increased solubility in water and alkalis and that part of the sulphur is oxidised to sulphurous acid, some of which is changed further to sulphuric acid. Bergen[6] concluded that the presence of acid promoted photo-chemical decomposition, whilst alkalis retarded it. Harris and Smith[8] agreed with this view. Some observers have noted destruction of epithelial scales.

In the experiments here described attention has been directed chiefly to the following points: (1) the destruction of epithelial scales and production of structural damage; (2) increased solubility in water and alkalis; (3) the nature of the acid formed, and the protective action of alkali and acid; (4) the effect of moisture and air.

Purified wool and wool containing known amounts of acid or alkali were exposed to the action of both ultra-violet light and light from a Fadeometer. The samples were spread out in thin layers and turned frequently (every hour). The time of exposure was 24 and 48 hours for ultra-violet and Fadeometer light respectively. The wool used was either Botany tops or Botany web. The results differed somewhat with the two kinds owing to the greater surface of the tops, and ultra-violet light produced a greater effect than Fadeometer light.

Experiments with Neutral Wool.—The wool was purified by the oleic acid method[9] and gave no reaction for acid or alkali. Hydrogen sulphide was given off during exposure, and a yellowish tint was produced. The exposed wool showed incipient damage of the epithelial scales and occasional fibres in which they had been destroyed. An increased affinity for Kiton Red and diazotised sulphanilic acid, indicating structural damage, was noted. These tests, however, do not establish de-amination. The sulphur was determined before and after exposure, a loss of 0·25 per cent. being found. The exposed wool wetted quickly in water and sodium hydroxide solution. After the wool had been for 12 hours in contact with water and with 0·1 *N* sodium hydroxide solution the losses in weight shown in Table I were recorded.

Table I

	Loss before exposure Per Cent.	Loss after exposure Per Cent.
Water	0·16	0·74
0·1 *N* NaOH	1·80	4·80

After exposure to both ultra-violet light and Fadeometer light the wool was strongly acid to methyl red and methyl orange, and this acidity was proved by extraction with alcohol to be due to sulphuric acid. No sulphurous acid could be detected.

The actual increase in acidity is shown in Table II.

Table II

	Acid before exposure Per Cent.	Acid after exposure Per Cent.
Tops	0·0	1·13; 1·12
Web	0·0	0·54

A sample of web treated with a very dilute solution of copper sulphate gave 0·98 per cent. of acid. The acidity throughout these experiments was determined both by distillation and by extraction with pyridine. The results agreed as a rule although there were occasional differences, due probably to uneven distribution of the acid.

A weighed quantity of the purified wool was dried in a silica flask and exposed, after the flask had been closed with a rubber bung, to ultra-violet light. The acidity after exposure was 0·74 per cent., indicating that the absence of moisture did not affect the production of acid. When the purified wool was exposed in a silica flask from which the oxygen had been removed no acid was formed but only hydrogen sulphide. It seems that the primary action of light is to break down the sulphur linkages, with production of hydrogen sulphide which, in presence of air, becomes partly oxidised to sulphuric acid. When some pure wool treated with potassium iodide and starch was exposed to ultra-violet light a bluish colour was quickly produced. It is probable therefore that ozone formed at the surface of the wool is the actual oxidising agent. It was shown by Trotman and Langsdale[1] that ozonised air produced some of the characteristics of "weathering," but the production of acid was not investigated.

THE RATE OF INCREASE IN ACIDITY.—Weighed portions of neutral web were exposed to the action of ultra-violet light for varying periods and analysed.

The results are given in Table III.

TABLE III

Hours exposed	Acid Per Cent.	Increase
12	0·34	—
24	0·44	0·10
36	0·64	0·20
48	0·74	0·10
72	1·02	0·28

ALKALINE WOOL.—Wool tops containing 0·38 per cent. of alkali as sodium carbonate and alkaline to phenolphthalein was exposed to the action of ultra-violet light for 24 hours.

After exposure it was acid to phenolphthalein but alkaline to phenol red. No damage could be detected microscopically, but faint indications were given with diazotised sulphanilic acid and Kiton Red. The colour was slightly yellow and the wool wetted quickly in water and alkalis. The results of analyses are given in Table IV.

TABLE IV

	Before exposure	After exposure
Alkali, per cent.	0·38	0·05
Sulphur, per cent.	2·99	2·99
Solubility in water, per cent. ..	1·04	1·40
Solubility in *N* NaOH, per cent.	2·24	3·02

Another sample of web containing only a little alkali (pH 7·5) gave after exposure 0·29 per cent. of acid. It appears that the acid formed is neutralised by the alkali, and that the presence of the latter exercises a protective action.

ACID WOOL.—Experiments were made with woollen web containing known amounts of sulphuric acid. In every instance the percentage of acid was increased by exposure (Table V). Hydrogen sulphide, but no sulphur dioxide, was given off during exposure. The loss of sulphur was determined in one experiment, and found to be 0·19 per cent. No damage could be detected microscopically. The exposed wool wetted rapidly in water and alkalis and gave faint reactions with Kiton Red and diazotised sulphanilic acid. The solubility in water was nearly the same as that of the alkaline wool (*viz.* 1·20 per cent.), but the solubility in $N/10$ sodium hydroxide solution increased to 6·2 per cent.

TABLE V

Acid before exposure Per Cent.	Acid after exposure Per Cent.	Increase
0·39	0·69	0·30
*0·41	0·74	0·33
0·74	1·23	0·49
1·17	1·72	0·55
1·49	1·99	0·50
2·05	2·64	0·59
5·88	6·35	0·47

* This sample was exposed to the Fadeometer for 48 hours. A second sample treated with a little copper sulphate gave after exposure 1·47 per cent. of acid.

DYED SAMPLES.—Samples of web were dyed in the usual way with an acid dyestuff in presence of Glauber's salt and sulphuric acid; they were then washed and dried. The acidity was determined on weighed portions before and after exposure to ultra-violet light. The results are given in Table VI.

TABLE VI

Dyestuff	Acid before exposure Per Cent.	Acid after exposure Per Cent.	Increase
Bordeaux B	1·76	2·17	0·41
Orange II	2·13	2·50	0·37
Diphenyl chlorine yellow FF (thiazol dyestuff)	2·25	2·65	0·40

SUMMARY.—The conclusions that may be drawn from these experiments are:

(1) Ultra-violet light causes some modification of the wool protein, which increases its solubility in alkalis, and, to a smaller extent, in water.

(2) Neutral wool is more susceptible to the action of light than either acid or alkaline wool, and acid wool is more susceptible than alkaline wool.

(3) The principal chemical change that takes place is liberation of hydrogen sulphide, part of which is oxidised by contact with air (or traces of ozone) to sulphuric acid.

(4) This sulphuric acid is of importance in connection with the determination of acid in woollen goods.

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1, REGENT STREET
NOTTINGHAM

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Generator for "Air-free" Carbon Dioxide (or Hydrogen)

BY S. HORWOOD TUCKER, M.A., D.Sc., A.R.C.Sc., F.I.C.

THE object in designing the generator here described was to obtain a supply of carbon dioxide of sufficient purity to be used for the micro-determination of nitrogen in organic compounds.

"AIR-FREE" CARBON DIOXIDE FOR NITROGEN DETERMINATION.—*Historical.*[1,2] —The Dumas method of determining nitrogen in organic compounds has undergone very little change since it was first introduced. In 1831 Dumas,[3] while attempting to improve Liebig's method, suggested that in making a *qualitative* test for nitrogen it might be removed from the combustion tube by means of carbon dioxide. In 1833[4] he adopted what is essentially the method still in use, *viz.* lead carbonate placed in the closed end of the tube was heated to generate carbon dioxide, which effected removal of air. After combustion of the substance the nitrogen produced was swept out, by renewed heating of the carbonate, and collected over potassium hydroxide solution. Since that time, except for the introduction of Schiff's azotometer,[5] the chief modifications in the method have been concerned with the carbon dioxide supply.

Thus, carbon dioxide has been produced (*a*) by heating the following substances in the closed end of the tube or in a separate tube: carbonates of lead, copper (Liebig, 1837), manganese (Simpson, 1853) and magnesium (magnesite), and the bicarbonates of potassium (Henry and Plisson) and sodium (Melsens, Erdman and Marchant, 1846; Breuer, 1937[6]); (*b*) by the action, in a separate apparatus, of acids on carbonates, *e.g.* chalk + sulphuric acid solution (Hoogewerff and van Dorp, 1882[7]) fused sodium carbonate (also a fused mixture of sodium and potassium carbonates) + sulphuric acid solution (Kreusler, 1885[8]), marble + hydrochloric acid solution (Hufschmidt, 1885[9]), conc. potassium carbonate solution + sulphuric acid solution (Blau, 1892[10]; Young and Caudwell, 1907[11]), potassium bicarbonate solution + sulphuric acid solution (Farmer, 1920[12]; Poth, 1930[13],

Lowe and Gutmann, 1932,[14] Reihlen, 1939[15]); (*c*) from liquid carbon dioxide (Ludwig, 1880[16]) and from solid carbon dioxide (Hershberg and Wellwood, 1937[17]).

One of the most convenient methods of preparing carbon dioxide is from marble and hydrochloric acid. For this purpose Kipp's apparatus has been universally used; many modifications of it have been suggested, *e.g.* by Kreusler, 1885,[8] p. 227, modernised by Schoeller, 1921[26]; Böck and Beaucourt, 1928[18]; Trautz and Niederl, 1931[19]; Berger, 1937.[20] A greatly improved generator was introduced by McCoy.[21] Objection has often been brought against the use of marble on the ground that it may contain occluded air. Accordingly, following the practice of Blau,[10] Young and Caudwell[11] used a conc. solution of potassium carbonate + dilute sulphuric acid (1 : 1 by vol.) in an apparatus modelled on one introduced by Thiele, 1899.[22] This type of apparatus suffered, however, from the serious defect that rubber stoppers were an integral part. (This will be referred to again later.) Poth[13] eliminated this defect by devising an all-glass apparatus, which was later improved in design by Lowe and Gutmann,[14] and by Reihlan.[15] They all used potassium bicarbonate solution + sulphuric acid (1 : 1 by vol.). (See also Bradley and Hale, 1908.[23]) Reihlan, 1939,[15] claimed to have prepared *air-free* CO_2, but covered the claim with the statement "Unter 'völlig luftfrei' wird dabei ein Kohlendioxyd verstanden, dessen Mikrobläschen im Azotometer durch eine etwa sechsfach vergrössernde Lupe bei gewöhnlicher Beleuchtung nicht mehr wahrgenommen werden können."

With respect to the use of marble, various attempts have been made to remove air which it is alleged to contain. Bernthsen[24] subjected it to reduced pressure, but Bradley and Hale[23] stated that occluded gases could not be removed from marble (magnesite and the like) "by subjecting the powdered mineral to the continued action of boiling water or of a vacuum." This was reiterated by Poth[13] and tacitly assumed by Reihlan.[15] Pregl and Roth,[25] however, as late as 1937, still recommended as source of carbon dioxide (for the micro-Dumas determination of nitrogen—for which a high state of purity is necessary) marble + hydrochloric acid, the marble being first etched with dilute hydrochloric acid, boiled in water for 10 minutes, and finally left under reduced pressure.

Since the object of this work was to produce a generator which would provide carbon dioxide sufficiently pure for micro-Dumas determinations, I was concerned with providing a *practically*, rather than a *completely*, air-free supply. I do not believe that the latter has ever been obtained, since even Reihlan[15] admitted that unabsorbed bubbles were discernible in the potash solution used for absorption of the carbon dioxide produced by his apparatus.

The chief defect in my opinion in many types of apparatus lies not in the use of marble but in the necessary use of rubber stoppers and connections. Many have found not only that carbon dioxide can pass through rubber, but that air can diffuse through it, even against pressure (Bradley and Hale[23]). On this ground alone one should question the accuracy of the claim of Young and Caudwell,[11] that their apparatus, set up as described, could consistently produce carbon dioxide containing only 1 part of impurity in 50,000; they do not describe how the small volume (0·1 ml.) of impurity and the large volume (5 litres) of carbon dioxide generated were measured.

If, in the apparatus described below, the ground-glass connections are replaced by vaseline-impregnated rubber stoppers* (Pregl–Roth,[25] p. 26), air leaks in, and after about a week is present in sufficient quantity to vitiate a micro Dumas nitrogen determination; but if the apparatus is in daily use leakage is not detectable.

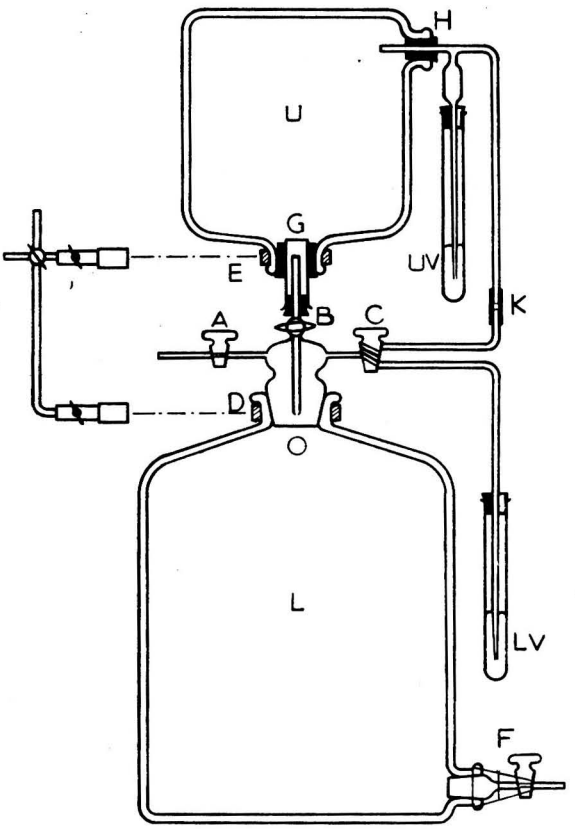

Fig. 1

DESCRIPTION OF "AIR-FREE" CARBON DIOXIDE GENERATOR.—The construction of the apparatus is shown in the diagram (Fig. 1). The lower glass vessel, L, is a Mariotte flask (5 litres) with ground-glass necks into which fit (at F) a glass tap (wired on) and (at D) a ground-glass fitting, ABC,† consisting of two one-way taps, A, B, and one two-way tap, C, the latter enabling connection to be made between the lower vessel, L, and either the upper vessel, U, or the lower mercury valve, LV. The tube to which tap B is attached ends (at O) in a small opening (1 to 1·5 mm. in diameter); it should not be a drawn-out nozzle. The glass

* The stoppers were immersed in vaseline contained in a beaker and kept at 80° C. for 10 minutes. The whole was then submitted to evacuation in a vacuum desiccator for about half-an-hour. After atmospheric pressure had been regained the operations were repeated, but evacuation was continued until no more bubbles rose from the stoppers. These were then left to cool under the vaseline at atmospheric pressure.

† Registered (Design No. 833,113) at H.M. Patent Office.

fitting, ABC, is attached to the upper, inverted Mariotte flask (U, 2 litres) by rubber stoppers and a glass tube (BG, 5 × 1·2 cm., made by cutting off the closed end of a Pyrex test-tube). The insertion of this tube, BG, enables the upper end of the acid-delivering tube, BO, to remain covered with acid, avoids the use of a rubber connection (between OB and U), and provides a flexible joint which at the same time is firm enough to hold the glass fitting, ABC, down in its socket, D. The upper vessel, U, is supported on the neck of the lower, L, by clamps (at E and D). The upper valve, UV, and the lower, LV, are test-tubes (13 × 1·2 cm.) containing mercury (3·5 cm. deep in UV and 4 cm. in LV). They are attached to the capillary-ended glass tubing by rubber stoppers provided with slit-openings, through which excess carbon dioxide can escape. Water on the surface of the mercury tends to lessen splashing and keeps the mercury clean. The rubber tubing, K, may be of any type, since leakage at this connection is of no consequence.

REAGENTS.—Carbon dioxide is generated from marble and hydrochloric acid, which is prepared by mixing the conc. acid with an equal volume of boiled-out water and boiling the mixture for 10 minutes to remove dissolved air. The marble in small chips (1 cm. long) is washed with hot water to remove dirt, and covered with dilute hydrochloric acid (200 ml. of conc. acid diluted with water to fill a Winchester quart). When the effervescence has ceased the liquor is poured away, and the etching process is repeated; but instead of the liquor being poured away, the whole (liquor + marble) is boiled for half-an-hour to remove adherent air and then cooled, this ensuring that the liquor covers the marble.

ASSEMBLY.—All taps and joints should be treated with thick grease, *e.g.* a rubber in vaseline mixture; vaseline by itself is ineffective. The bottom tap (F) is securely wired in—it need never be removed again, even when the apparatus is re-charged. The 5-litre vessel (L) is filled with the marble and covered with the spent liquor in which it was boiled or with boiled-out water. The remainder of the apparatus is fitted up as shown. The upper vessel (U, with B closed) is then filled with the prepared acid, a condenser adapter or funnel + rubber tubing being utilised (at H).

CLEARING OUT AIR.—Taps A and C are closed, F is opened and then B. Acid attacks the marble and the liquor in L is expelled through F; excess of carbon dioxide will escape through the acid in U. (Care must be taken that this evolution is not too violent; if it becomes so, B is closed, A and C (to UV or LV) are opened and F is closed.) L is then evacuated through F by means of the water-pump.* F is closed and B opened. The evacuation is twice repeated. If at any time the pressure (registered by UV) above the acid becomes so reduced that acid ceases to flow through B, pressure may be re-developed by opening C *momentarily* (so as to connect L with U). It is not usually necessary to resort to this expedient, since, normally, bubbling back (through B) is sufficient to maintain the pressure (registered by UV); but in order to remove the last traces of air from the acid (Farmer[12]) the operation described above (opening C momentarily while B is

* Although no implosion has ever occurred, it is advisable, as a precautionary measure, to carry out this operation behind the screen of the draught cupboard.

The expedient, adopted by Reihlan,[15] of leaving his apparatus standing full of carbon dioxide for several days to allow air to be displaced from glass surfaces does not seem to be necessary with the generator now described, after the above evacuation.

permanently open) should be repeated a number of times. The process further serves to displace air from above the acid (in U) and thus preserve it permanently from re-contamination. C is opened and air is driven from the tube leading to the valve LV. If the apparatus is not yet quite free of air, evacuation may be repeated, or L may be flushed out by closing A and C, and then opening B and F alternately. The generator is left with A and B closed and C open to LV. When necessary spent liquor is removed by closing A and C and manipulating B and F as just described.

MANIPULATION.—To obtain a supply of carbon dioxide, close C and open B. Then, since the pressure in L is almost invariably greater than that in U, bubbles of carbon dioxide will escape upwards through B and through the mercury in UV. Pressure will thus be established. If this does not take place, open C (to UV) *momentarily* (*cf.* above). Open A to deliver carbon dioxide. With B fully open the flow of acid is usually so great that a large excess of carbon dioxide is produced (and escapes through UV). Accordingly, B is adjusted so that the bubbling back (intermittent) is only sufficient to maintain the head of mercury in UV. When so set the generator works automatically.

When the supply of carbon dioxide is no longer needed A is closed and the generator may be left with B adjusted as above. No acid will enter L unless B is open too wide, or the temperature of the environment changes considerably. In any event only a wastage of reagents occurs. This is a convenient condition in which to leave the generator when it is in intermittent use; carbon dioxide is then immediately available and controllable by manipulation of tap A only. However, if the generator is left unused for longer than a day it is economical to close B and open C so as to connect L with the valve LV. In this condition it may be left in a perfectly air-tight condition for any length of time; when again required, unimpaired carbon dioxide will be at once obtainable.

TESTING THE GENERATOR.—Carbon dioxide was passed into a micro-azotometer for 3 hours, bubbling being continuous and regular without any adjustments being intermediately required. With 5 bubbles passing per second, 0·010 ml. was collected in 3 hours, *i.e.* 0·0006 ml. in 10 minutes. In the micro Dumas nitrogen determination carbon dioxide is passed at a much slower rate than 5 bubbles per second, and for about 10 minutes only. Hence the above supply would not give the lowest observable amount (0·001 ml.) of unabsorbed gas during the determination.

To gain an *approximate* idea of the volume of carbon dioxide passed in 3 hours, the apparatus was set up as described above, and when the stream into the azotometer was 5 bubbles per second, the potassium hydroxide solution was run off and replaced by water. The carbon dioxide was then collected in an auxiliary receiver; it measured 1045 ml. in 3 hours. Hence 1045 ml. contained 0·010 ml. of unabsorbed gas, *i.e.* less than 1 part of unabsorbed gas in 100,000 parts of carbon dioxide.

ADVANTAGES OF THE GENERATOR.*—(1) A practically air-free supply of carbon dioxide sufficiently pure for micro nitrogen determination is obtainable.

* The generator (without mercury) may be obtained through the usual retailers, from Messrs. G. W. Flaig & Sons, Ltd., 57, Hatton Garden, London, E.C.1; price £3.

(2) A continuous or discontinuous supply of carbon dioxide at a regular and adjustable pressure is obtainable by manipulation of one tap only.

(3) The total supply of carbon dioxide is limited only by the size of the marble-container; which may be of any desired size without alteration of the small area of ground glass surfaces. With the 5-litre size, several hundred micro-analyses may be carried out from one charge.

(4) There are no rubber connections through which air may enter and vitiate the carbon dioxide.

(5) The generator may be set aside for an indefinite period without wastage of reagents or lowering of quality of the carbon dioxide taking place.

(6) The same generator may be used for the preparation of *air-free hydrogen* from granulated zinc and hydrochloric acid (prepared as described above).

I am indebted to Mr. J. M. L. Cameron of this Department, who has greatly assisted me with suggestions and in testing the apparatus.

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March, 1939

The Volumetric Determination of Organic Picrates and Picrolonates with Methylene Blue

By ADOLPH BOLLIGER

A NUMBER of nitrophenols and organic acids combine with methylene blue to form salts which are only slightly soluble in water but more soluble in chloroform. By applying this property a titration method has been evolved for the micro-determination of suitable nitrophenols and aromatic acids, as well as of their salts and addition compounds.[1,2] Of the nitrophenolates tested, picrates are the most suitable substances for titration with methylene blue, and the rapidity, as well as the accuracy, of this procedure warrants a description of the determination of the organic picrates that are frequently prepared. Picrolonates are also readily determined by this method if certain precautions are taken.

PRINCIPLE.—The picrate is titrated with 0·001 *N* methylene blue solution, and the methylene blue picrate or picrolonate formed in the titration is extracted with chloroform from the aqueous layer. Since methylene blue is practically unextractable with chloroform, it is possible to detect the end-point by the colour of the aqueous layer, the titration liquid acting as indicator.

REAGENT.—Approximately 0·001 *N* methylene blue solution. First, an approximately 0·01 *N* stock solution is prepared by dissolving 3·8 g. of chemically pure methylene blue in water and diluting the solution to 1 litre. (We used methylene blue "Merck, medicinal, chemically pure, free from chloride of zinc, $C_{16}H_{18}N_3SCl + 3H_2O$"); 0·001 *N* methylene blue solution is obtained from the stock solution by dilution. These solutions, when preserved with a few ml. of chloroform and kept in a dark bottle, remain sufficiently stable for several months. The 0·001 *N* solution of methylene blue is standardised against a 0·001 *N* solution of chemically pure picric acid by the titration method described below.

METHOD.—From 1 to 15 mg. of the substance to be titrated is weighed on the micro-balance and used as such, or an aqueous solution containing a known amount of the substance may be prepared. If the substance is not readily soluble in water, sufficient pyridine may be added to bring about complete solution, or the substance may be dissolved in chloroform. The solid substance, or a measured amount of the solution containing approximately 1 to 15 mg., is transferred to a cylindrical separating funnel containing a few ml. of water and about 20 ml. of chloroform. Aqueous methylene blue solution of known strength (approx. 0·001 *N*) is run in from a burette, and the funnel is shaken to extract the methylene blue picrate from the aqueous layer by means of the chloroform already present. The chloroform layer, which separates very rapidly, assumes a green colour, while the yellow colour of the aqueous layer diminishes correspondingly. The titration is continued, the chloroform being renewed when it is saturated with methylene blue picrate or when the aqueous layer has assumed the green tint, although not the intensity of colour, of the chloroform layer. In the latter event, the aqueous layer, after extraction with fresh chloroform, becomes pale yellow. If a pale yellow colour of the aqueous layer has been reached, the methylene blue is added,

drop by drop, and the chloroform is frequently renewed. Theoretically, the end-point is reached when the aqueous layer has become completely colourless and when no more methylene blue picrate can be extracted from the aqueous layer. For practical purposes, however, the end-point is not considered to be reached until one drop of methylene blue changes the almost colourless solution to a faint but distinct blue which completely resists further extraction with fresh chloroform.

After a little experience it is easy to recognise the end-point by observing the changes in the aqueous layer, as described. For some observers, it may be somewhat easier to detect the end-point by observing the colour of the chloroform layer. As already mentioned, the yellow colour of the aqueous layer diminishes during the progress of titration, whilst the chloroform layer turns correspondingly deeper green. For the modified method of observing the end-point, the chloroform is renewed after every drop of the methylene blue solution as soon as the end-point is approached. The fresh lots of chloroform have to be of equal volume (for example, 10 ml.), and they are kept separately in a test-tube after each extraction. As long as picric acid is present in the aqueous layer, the chloroform extracts will be distinctly greenish. The end-point is reached when a distinct diminution and alteration of the colour of the extract from greenish towards a very faint blue occurs. This last sample is of the same colour as that obtained in a blank experiment containing about the same amount of water plus one drop of methylene blue solution extracted with a similar amount of chloroform. If, for some reason, the end-point has been overstepped, one can titrate back with 0·001 *N* picric acid solution.

Organic picrolonates are titrated in the same manner. However, one has to consider the fact that picrolonic acid is much less soluble than picric acid and that organic picrolonates frequently are very sparingly soluble. Therefore, it is frequently necessary to dissolve such picrolonates beforehand, in order to avoid losses during the process of titration. This is sometimes best effected by decomposing the sparingly soluble picrolonate in a boiling aqueous solution of lithium carbonate. The lithium salt of picrolonic acid formed is readily soluble. However, no considerable excess of lithium carbonate must be present, otherwise the results obtained will be too high. If an excess of lithium carbonate is present it is necessary, after the solution has been transferred to the separating funnel and immediately before the titration, to acidify slightly with sulphuric acid and to neutralise the excess of sulphuric acid by the addition of calcium carbonate.

The following table gives results obtained by titrating picrates and picrolonates with methylene blue:

(*A*) *Picrates.*	Amount analysed mg.	0·001 *N* methylene blue used ml.	Picric acid	
			Found Per Cent.	Calculated Per Cent.
Nicotine picrate	4·68 (solution)	15·1	73·9	73·9
		15·0	73·4	
Naphthalene picrate	7·770 (solid)	21·6	63·7	64·1
Acenaphthene picrate	8·85 (solution)	23·1	59·7	59·8
		22·9	59·3	

(*B*) *Picrolonates.*	Amount analysed mg.	0·001 *N* methylene blue used ml.	Picrolonic acid Found Per Cent.	Picrolonic acid Calculated Per Cent.
Nicotine picrolonate	4·171 (solution)	12·2	77·2	76·5
p-Bromoaniline picrolonate	11·56 (solid)	26·6	60·8	60·5
		26·8	61·2	
Dibenzylamine picrolonate	9·18 (solution)	20·1	57·7	57·3

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April, 1939

Notes

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

THE DETERMINATION OF COPPER

THIS method for the determination of copper in alloys of the Duralumin type was designed primarily to supplement the electrolytic method. It has proved to be much quicker than the latter, while retaining an accuracy well within the limits usually required for general routine work, and it requires no special or expensive apparatus. Indeed, a minimum of apparatus is used, as all the operations are conducted in one and the same beaker.

It is a variation of the iodimetric procedure but involves no separations. Iron and manganese, which interfere quantitatively in the oxidised state, are reduced by excess of sodium sulphite, which excess is destroyed by boiling, and re-oxidation is permanently inhibited by the presence of the excess of aluminium sulphate.

PROCEDURE.—One g. of the drillings is placed in a covered 350-ml. Pyrex conical beaker and dissolved in 35 ml. of the following acid mixture:—water, 6; conc. hydrochloric acid, 3; conc. nitric acid, 3; conc. sulphuric acid, 1·7 vols. The solution, still under cover, is boiled gently for a few minutes and then evaporated rapidly to dryness on the hottest part of a hot plate. The white cake, after cooling somewhat, is covered with about 100 ml. of boiling water, and 5 ml. of a freshly prepared 4 per cent. solution of sodium sulphite hexahydrate is added together with a few mg. of finely powdered quartz. The solution is boiled steadily for 10 to 15 minutes, and then cooled in running water. The cover is rinsed into the beaker, and about 2 g. of solid potassium iodide (measured in a spoon) are added. The solution is shaken to dissolve the reagent and titrated at once with standard sodium thiosulphate solution (1 ml. = 0·0025 g. of Cu), starch solution being used as an indicator. If the titration is conducted at 35–40° C., the end-point is sharp.

The following are typical comparative results obtained by the electrolytic and iodide methods:

Electrolytic Per Cent.	Iodide Per Cent.	Difference
3·95	3·90	0·05
4·33	4·33	nil
4·00	4·02	0·02
4·50	4·50	nil
4·89	4·85	0·04
4·50	4·51	0·01
4·61	4·58	0·03
4·69	4·62	0·07
3·39	3·38	0·01
3·20	3·15	0·05
3·52	3·58	0·06
4·50	4·45	0·05
4·54	4·53	0·01
3·21	3·15	0·06
4·08	4·15	0·07
4·19	4·25	0·06
4·17	4·18	0·01
4·39	4·35	0·04
4·30	4·30	nil

In 53 determinations on samples in which the copper-content ranged from 3·19 to 4·89 per cent. the discrepancies between the results obtained by the two methods were as follows:—In 2, 0·07; in 5, 0·06; in 5, 0·05; in 10, 0·04; in 9, 0·03; in 8, 0·02; in 8, 0·01; in 5, nil.

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April, 1939

SCREENED METHYL ORANGE

THE use of xylene cyanol FF to "screen" the colours given with methyl orange indicator was first mentioned by Hickman and Linstead (*J. Chem. Soc.*, 1922, 2502; Abst., ANALYST, 1923, **48**, 89) and commented on favourably by A. D. Mitchell in his lecture to the Institute of Chemistry in 1934. A very sharp end-point at *p*H 3·8 could thus be obtained.

On testing a bought sample of screened methyl orange in an ordinary HCl—NaOH titration, I obtained results that were unsatisfactory and showed no advantage over those with unscreened methyl orange, and the end-point was not sharp. On making up some screened methyl orange from solid xylene cyanol FF and solid methyl orange in the proportions given in Hickman and Linstead's original paper, I again obtained similar unsatisfactory results.

I found, however, that by using the same solid methyl orange with solid xylene cyanol FF from another source of supply, the result was entirely satisfactory, and the end-point at *p*H 3·8 was very sharp. Good results were also obtained with the first-mentioned sample of xylene cyanol FF if I used it in about double the proportion per unit of methyl orange, namely, 2·8 g. of xylene cyanol FF, instead of 1·4 g. per 1 g. of methyl orange, in 500 ml. of 50 per cent. alcohol.

It would thus appear that samples of xylene cyanol FF are liable to vary considerably. The poor sample was of a deep indigo colour in the solid state, but when dissolved alone in 50 per cent. alcohol it gave a blue solution paler than that of a solution of the good sample at the same concentration. In the solid state the good sample was greyish-blue.

The poor and good samples of xylene cyanol FF gave the following respective colour changes with screened methyl orange:

*p*H	Poor sample	Good sample
3·1	Red	Purple
3·6	Reddish-brown	Greyish-purple
3·8	Yellow-brown	Grey
4·0	Yellow-brown	Greenish-grey
4·2	Yellow	Green

This note may be of value to analysts, as an indicator giving a sharp end-point at *p*H 3·8 is useful in many titrations.

R. FARADAY INNES

THE BRITISH LEATHER MANUFACTURERS' RESEARCH ASSOCIATION
18–20, ST. THOMAS' STREET
LONDON, S.E.1

April, 1939

A USEFUL AID TO SUGAR ANALYSIS

IN recent years the Lane and Eynon method of sugar determination has won deserved popularity amongst food analysts. From our practical experience, we are of the opinion that once the technique of the method has been thoroughly grasped, and provided that the conditions of carrying out the test are followed exactly, it is possible to obtain a series of successive titrations which do not differ from each other by more than 0·05 ml. When, therefore, it was observed that this order of accuracy was obtainable, it then became necessary to take one of three steps in order to obtain a result in keeping with this standard of accuracy.

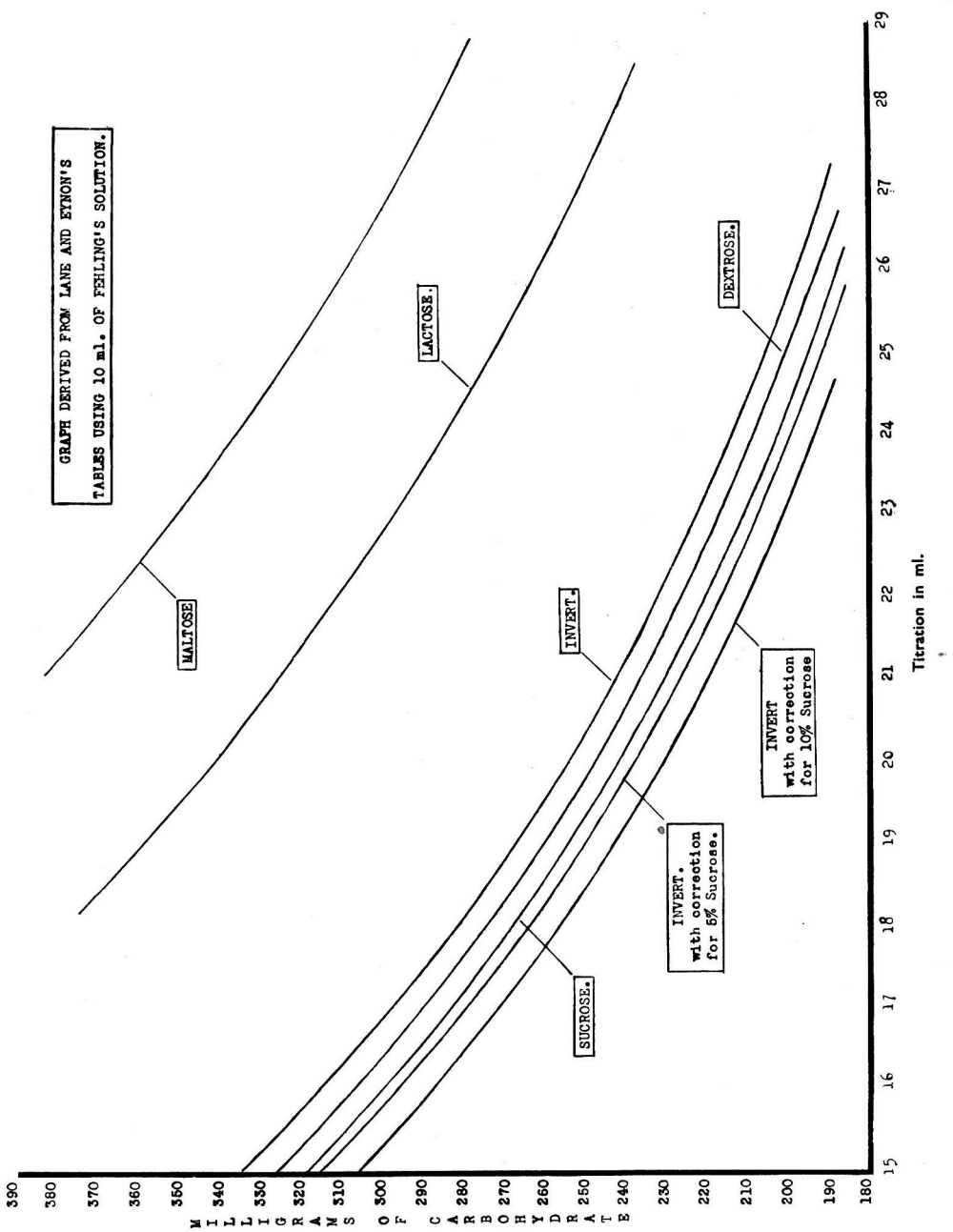
(1) To extend more fully the Lane and Eynon tables, so that readings would be available for every 0·05 ml. between 15 and 50 ml. This would involve the recording of over 700 readings for each particular sugar, and the idea was therefore discarded on the grounds of being impracticable.

(2) To apply the factor that is given in the tables for the nearest whole number of ml. This offers no serious objection, except in so far as the analyst would be continually dealing with a different factor in every calculation.

(3) To prepare a graph by interpolation of the Lane and Eynon tables in such a way that the milligrams of carbohydrate in 100 ml. of solution would be given directly for any particular titration. We formed the opinion that if the idea underlying this last method were investigated, then once the necessary graph had been prepared, a ready means would be available for calculation of all Fehling determinations without the introduction of any troublesome factors.

We outline below a description of the type of graph that we have prepared and have found of considerable use during the past twelve months.

The graph was constructed from the Lane and Eynon tables, by plotting milligrams of carbohydrate per 100 ml. of solution against the results of Fehling titrations for most of the common sugars. The linear dimensions were approximately 25 in. × 19 in. The graph was mounted on a wooden framework provided with a sliding cursor also made of wood. This cursor had a printed titration scale running along the whole of its length, and was so constructed that it could slide in a direction parallel to the longitudinal axis of the graph.


GRAPH DERIVED FROM LANE AND EYNON'S
TABLES USING 10 ml. OF FEHLING'S SOLUTION.
MILLIGRAMS OF CARBOHYDRATE
Titration in ml.
MALTOSE
LACTOSE.
INVERT.
INVERT. with correction for 5% Sucrose.
SUCROSE.
INVERT with correction for 10% Sucrose
DEXTROSE.

To use the graph in actual practice, the following procedure is recommended:—Begin by obtaining the Fehling titration by the Lane and Eynon method, and then move the cursor in an upward direction until the figure on the scale attached, corresponding with that obtained by titration, cuts the curve of the particular sugar being determined. Then, if the eye is cast along the cursor to the point where it cuts the vertical axis of the graph, the milligrams of carbohydrate present in 100 ml. of solution may be read directly.

In the accompanying diagram it will be noted that a curve for sucrose is also indicated. This, of course, was prepared by multiplying the invert sugar figures by 0·95. We wish to point out that if the number of sugar determinations carried out in any particular laboratory is sufficiently large to warrant the use of this graph, it would have to be constructed to individual requirements and one or two minor alterations carried out. Thus the graph described above covers only a range of titrations from 15 to 29 ml., although Lane and Eynon have published factors for titration from 15 to 50 ml. Also, we use only 10 ml. of Fehling's solution for titration, but a similar graph can be prepared for use with 25 ml. if required.

We wish to record our thanks to Mr. A. S. Houghton and to Messrs. Fuller's, Ltd., for permission to publish this note.

R. L. KENNY
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HAMMERSMITH, LONDON, W.6

March, 1939

Department of Scientific and Industrial Research

MICROBIOLOGY IN THE PRESERVATION OF THE HEN'S EGG*

THE Report summarises existing knowledge on the avian egg in general, with special reference to the hen's egg, and discusses the subject under the following divisions:—(1) The anatomy and physiology of the bird and developing ovum, especially in connection with possible microbial activity. (2) The structure and chemistry of the egg in their relation to the micro-flora of the environment. (3) Consideration of factors operating during storage in so far as these are relative to the microbiology of the subject.

MICROBIOLOGY OF THE EGG DURING OVI-DEPOSITION.—For many years the view has been promulgated that, whilst the infertile egg may be bacteriologically sterile, the fertilised egg frequently is not. The general conclusion, drawn from a consideration of the published work, is that it is possible that, although fertilisation apparently does not lead to increased bacterial infection, it may set going a chain of enzymic and physiological changes rendering the fertilised ovum more susceptible to bacterial decomposition, but there appears to be no scientific evidence on this question. Experimental work has shown that the bird displays a high degree of resistance towards bacterial infection and, in particular, that even when the ovary does become infected, the infection is not readily transferred to the egg. The balance of experimental evidence does not support the trade view that infection of the egg increases with the age of the hen.

MICROBIOLOGY OF THE EGG AFTER LAYING.—The number of "rots" is much higher, as would be expected, with eggs with dirty shells than with clean ones, but washing, or cleaning the shells in any way, is useless and in fact increases the rate of deterioration. The reason for this has apparently not yet been definitely established. It may be that washing removes the coating from a sound egg and

* Food Investigation. Special Report No. 47. By R. B. Haines, D.Sc., Ph.D. Pp. 65. With 3 plates and 5 figures. H.M. Stationery Office, York House, Kingsway, London, W.C.2. March, 1939. Price 2s. 6d. net.

thus renders it more susceptible to subsequent bacterial infection, or it may assist the passage of the bacteria already on the shell into the egg. Again, the coating of mucin deposited on the newly-laid egg may itself have a germicidal action. These are points that require further investigation.

Since the spoilage of eggs is due above all to microbiological activity, it follows on general grounds that all possible steps should be taken to reduce the infection at all points of handling. Hay and straw in general being known to harbour great numbers of micro-organisms, clearly the egg should be kept out of contact with them as far as possible.

There are substances in egg-white which are inimical to bacteria, but, rather surprisingly, the white of really fresh eggs has a weaker germicidal action than that of older eggs. The explanation suggested is that as the egg gets older it loses carbon dioxide and the white thus becomes so alkaline that most organisms cannot grow in it. Moreover, there is a substance in the white of eggs (and also present in human tears), which has the property of "lysing" the organisms by dissolving them. With one variety of bacteria, a dilution of one of egg-white in two millions produces complete lysis of a thick suspension of the organism in three hours at a temperature of 113° F., and a dilution as low as one in sixty millions has a definite effect.

A number of requirements must be fulfilled before the micro-organisms can elaborate the substances, *e.g.* enzymes, which cause deterioration. For example, even the most active organisms require the presence of simple compounds before they can begin to grow, and must synthesise their enzymes before the decomposition of protein can begin. Sown into solutions of purified proteins, they die. Hence the amount of decomposition occurring in eggs before contaminating bacteria have access to them must have a great influence upon the ease with which the organisms grow and effect further decomposition. This, in turn, presumably depends on the rapidity with which the eggs are gathered and cooled to a temperature sufficiently low to minimise enzymic changes.

In the rotting of eggs caused by fungi, when the fungus penetrates as far as the yolk the yolk-membranes may be ruptured, and once this stage is reached, luxuriant growth of the fungus occurs. Rotting takes place rapidly in eggs with "sided yolks," and it is assumed that this is due to infection from the outside of the shell being able to penetrate more or less directly into the yolk, where it flourishes.

MICROBIOLOGY OF THE EGG DURING STORAGE.—The Report describes the commonest deleterious flavours or odours in stored eggs as "mustiness," "fishiness" and "cabbage-water" in the white, and a "strong" flavour in the yolk. The first three of these are micro-biological in origin. The cause of the last is not always clear, but it has been suggested that a "strong" or "storage" taste appearing after about seven months' storage is due to the absorption of odours from the surroundings, especially from straw-board fillers.

Briefly, the methods for reducing the spoilage of eggs by the activity of micro-organisms during storage are as follows:—hygienic production and handling, coupled with strict elimination of all inferior material; preservation of the natural defences of the egg; control of temperature and of humidity, and the use of gases, such as carbon dioxide, or of other means, such as sealing the pores of the eggs, for retarding the growth of the organisms.

Eggs to be cold-stored should be cooled as rapidly as possible to temperatures near 0 °C. This eliminates the possibility of "blood-heat" organisms multiplying, which means that the incidence of the most potent form of black rotting, due to *Proteus* bacteria, will be minimised. However, not only must the egg be rapidly cooled to, and kept near, 0° C., but adequate precautions must also be taken when it comes out of store. *Proteus* bacteria, although partially or completely prevented from developing during storage at 0° C., do not necessarily die under

these conditions, and the slow chemical and physical changes occurring during prolonged storage are likely to render the egg all the more easily attacked by surviving rot-producing organisms when the temperature is again brought within the range at which they grow. Ideally, therefore, cold-stored eggs should be used immediately on removal from storage. The high incidence of black rot occasionally found in imported eggs is almost certainly due to the eggs being allowed to stand about at too high a temperature after removal from storage.

The result of work upon the control of humidity to avoid the possibility of fungal spoilage indicates that the humidity of the store should not exceed 80 to 85 per cent. Dealing with other methods for reducing the growth of micro-organisms, the Report states that methods of storage depending on the exclusion of air, which prevent the growth of organisms needing oxygen, have been developed in a variety of ways. In the dry packing of eggs, salt, lime, bran, sawdust, ashes, sand and sulphur have all been used. None of these packings is completely successful in its objectives. Sealing with wax, with aluminium soap, or by immersion in water-glass is much better. The last method works well, eggs having been kept in good condition for several years by storage in waterglass. Its drawback, commercially, is the bulk of the packing material. Before being sealed with wax, the eggs are frequently evacuated, and the wax may be applied hot. Partial sterilisation, with better sealing of the pores, is thus accomplished.

Use of Carbon Dioxide.—The Report gives considerable attention to the use of carbon dioxide in the storage of eggs. Commercial methods for the gas storage of eggs, in which carbon dioxide has been used in conjunction with cold storage, have been developed by the Low Temperature Research Station at Cambridge. These are described in Food Investigation Leaflet No. 8 (The Cold Storage and Gas Storage of Eggs) copies of which are obtainable gratis on application to the Secretary, Department of Scientific and Industrial Research, 16, Old Queen Street, Westminster, S.W.1 (*cf.* ANALYST, 1939, 281).

BIBLIOGRAPHY.—The Report concludes with a detailed bibliography of six pages containing 158 references.

U.S.A. Commercial Standards for Disinfectants

THE National Bureau of Standards of the United States Department of Commerce has approved commercial standards for a number of disinfectants. The specified requirements include the following:

LIQUID HYPOCHLORITE DISINFECTANT, DEODORANT AND GERMICIDE.—The available chlorine present must be not less than 2·5 per cent., and the amount shall be clearly stated on the label. The loss in the available chlorine-content shall not exceed 10 per cent. when the disinfectant is stored in a cool, dark place at a temperature not exceeding 20° C. (68° F.). The preparation must be miscible in all proportions with water of zero hardness, and shall be ready for dilution when delivered.

PINE OIL DISINFECTANT.—This shall be the product of pure steam-distilled pine oil (of which it must contain not less than 60 per cent. by weight) mixed with an emulsifying agent. It shall remain clear and homogeneous under normal and reasonable conditions of storage. It must not contain more than 10 per cent. of water. The phenol coefficient, which shall be determined against *B. typhosus* by the Federal Food and Drug Administration method, must be stated on the label. The preparation shall not contain kerosene or other petroleum distillates. When 5 parts are diluted with 95 parts of water of zero hardness at 20° C. it shall form a stable emulsion, which shall show no sign of floating oil after it has stood for at least 24 hours.

COAL TAR DISINFECTANT (EMULSIFYING TYPE).—This shall consist of not less than 65 per cent. by weight of coal tar oils and acids, and an emulsifying agent, and shall not contain more than 10 per cent. of water. It must not contain kerosene or other petroleum distillates. The phenol coefficient, determined against *B. typhosus*, must be stated on the label. The product must form milky emulsions when diluted with water of zero hardness at 20° C. in the ratio of 5 parts of disinfectant to 95 parts of water for disinfectants with a phenol coefficient of 10 or under; or in the ratio of 2 parts of disinfectant to 98 parts of water for disinfectants with a phenol coefficient exceeding 10. These emulsions shall show not more than a trace of floating oil when stored for five hours at room temperature. There must be no sign of naphthalene crystallisation after 12 hours at 0° C. The preparation must contain less than 5 per cent. of benzophenol.

CRESYLIC DISINFECTANTS.—The product shall be made from that portion of coal tar known as "tar acids," and a soap derived from rosin acid and/or fat of vegetable origin. It shall contain not less than 50 per cent. of tar acids, as determined by the U.S.P. XI method for the assay of cresol in the saponated solution of cresol. It shall not contain more than 25 per cent. of inert ingredients (water plus glycerin, if any). The phenol coefficient, as determined against *B. typhosus*, must be stated on the label. It must contain less than 5 per cent. of benzophenol. When mixed with water of zero hardness at 20° C. it must yield a clear solution within the concentration range of 1 to 4 per cent. Such solutions, when kept in close containers, shall remain either practically clear or become only slightly opalescent when allowed to stand for 24 hours at 20° C. away from direct light. When kept at 0° C. for three hours the preparation shall not show any separation of soap.

HOUSEHOLD INSECTICIDE (LIQUID SPRAY TYPE).—The killing power shall be determined by the official Peet-Grady method in conjunction with the official test insecticide of the National Association of Insecticide and Disinfectant Manufacturers, Inc. The product shall have a flash-point not lower than 125° F. when tested in the Tagliabue closed cup.

U.S. Department of Commerce
National Bureau of Standards

MICROSCOPIC METHODS USED IN IDENTIFYING COMMERCIAL FIBRES*

THIS publication is a compilation of methods collected during a survey of the literature of the subject. All have been tried and found useful, and their relative merits are discussed. A bibliography of 48 references is included.

PAPER FIBRES

PREPARATION OF THE SAMPLE.—(1) *Federal Specification Method* (General Specification, UU–P–31, Sect. IV, Pt. 5, March 1, 1932).—A piece, about 5 mm. square, is cut from each of 10 sheets representing the delivery, and the pieces are boiled with 20 ml. of 2 per cent. sodium hydroxide solution and washed well with water. They are then rolled into a ball with the fingers and shaken in a test-tube with water until the fibres are completely separated. About 5 ml. of the suspension is transferred to another test-tube and again shaken with water, and as small a quantity of the fibres as can be taken up with a needle or with forceps is then placed on a microscope slide, and the water is removed by means of absorbent paper.

* Circular C.423, by T. M. Plitt. Pp. 26. Issued March 14, 1939. Superintendent of Documents, Washington, D.C., price 10 cents.

(2) *T.A.P.P.I. Method* ("Tentative and Official Testing Methods—Recommended Practices—Specifications," *Tech. Ass. of the Pulp and Paper Ind.*, 1936, T 401, m 36).—This is similar, except that the total area of samples should not be less than 6 sq. cm.; 0·5 per cent. alkali is used, and the fibres are transferred to the slide as a suspension which is subsequently dried in an air-bath; a 200-mesh sieve is used to separate the fibres from the alkali.

(3) *Short Method.*—The sample is boiled for 1 minute in 2·5 per cent. alkali, and the fibres are loosened first with the fingers and subsequently by stirring in an excess of water. A pipette is used to transfer the suspension to the slide, and the excess of water is removed with absorbent paper. In addition to rapidity this method has the advantage compared with (1) and (2) that there is no preferential selection of longer fibres by the needle or mesh, respectively.

QUANTITATIVE ANALYSIS.—(1) *Federal Specification Method.*—The whole of the surface of the prepared slide is systematically scanned under the microscope, at least 300 fibres being counted.

(2) *T.A.P.P.I. Method.*—A magnification of not less than 100 diameters is recommended, and a description is given of the preparation of a cross-line disc from two silk threads, which are cemented to a cover-slip which is fixed centrally under the diaphragm of the eyepiece of the microscope. The fibres are counted at various points in a straight line, twice lengthwise and four times across the slide, each line of observation being started at a different point; at least 25 different fields comprising 600 fibres, on 3 slides, should be counted. The fibres are recorded by counting each fibre as it passes under the intersection of the cross-lines, regardless of its size. Results are reported (as the percentage of the particular fibre present) to the nearest 5 per cent.

STAINS.—(1) *Herzberg General Stain.*—The Federal Specification and Merritt modification are given. For the latter (*Paper Trade J.*, 1922, **75**, 91TS), which is preferred, exactly 25 ml. of water are shaken with 50 g. of dry zinc chloride, and the sp.gr. is adjusted to 1·8 at 28° C. The solution is transferred to a tall cylinder, and the hydrometer, thermometer and containing-vessel are washed with about 5 ml. of water, the difference between this volume and 12·5 ml. being used to dissolve 5·25 g. of potassium iodide and 0·25 g. of iodine. The solutions are mixed well and placed in the dark, and on the next day all the clear liquid except a layer 4 cm. above the sediment, is transferred in a pipette to a black bottle; a scale of iodine is then added.

A correct stain should give the following colours:—rag, cotton, linen or hemp, wine-red or brownish-pink; chemical wood, adansonia, bleached straw and jute, dark blue; mechanical wood, unbleached jute or straw, lemon yellow; esparto, bluish or reddish; manila, varying shades (blue to yellow). The colour differences, however, are only to be regarded as a supplementary aid to the identification of fibres by their characteristic markings.*

(2) *Sutermeister General Stain* (T.A.P.P.I. Methods, *loc. cit.*).—The moistened fibres are stained with a solution containing 1·3 g. of iodine and 1·8 g. of potassium iodide in 100 ml. of water for 1 minute, the excess of stain is removed, and the fibres are treated with a clear, saturated solution of calcium chloride. Cotton, linen, hemp, and ramie appear red or brownish-red; bleached soda pulps from deciduous woods, dark blue; bleached and well-digested unbleached sulphite wood, blue or reddish violet; jute, manila, hemp and lignified unbleached sulphite wood, green; mechanical wood, yellow. An advantage of this stain is that it is not so dark as to obscure the markings on the fibres.

* Experience has shown that this procedure does not always give the same results; in particular, it is often difficult to distinguish rag and chemical wood fibres by their colours. It has been found desirable to test the stain on fibres of known origin, and to add more zinc chloride if the rag appears too blue or more iodine if the wood appears too red.—J. G.

(3) *Bright's Stain* (Federal Specification, *loc. cit.*).—A mixture of 10 ml. each of 2·7 per cent. ferric chloride ($FeCl_3$, $6H_2O$) solution and 3·29 per cent. potassium ferricyanide solution (filtered) is used to stain the moistened fibres for 20 minutes at 20° ± 1° C. The slide is then dipped into water six times, dried and immersed for 20 minutes in a fresh filtered solution of 3 g. of crude benzopurpurine-4B conc. in 500 ml. of cold water. It is finally washed and dried. Bleached fibres, or fibres practically free from lignocellulose, appear red; unbleached or lignified fibres appear blue.

In the Kantrowitz-Simmons modification (U.S. Govt. Printing Office, *Tech. Bull.*, No. 20, 1934) the procedure is shortened by the use of a 0·5 per cent. solution of the dye in warm 50 per cent. alcohol. This increases the amount of dye dissolved, and the staining-time is only 2 minutes. If the fibres are suspended in the stain, the period of the first staining may be reduced to 1 minute.

(4) *Klemm's Stain.*—The slide is placed in a saturated solution of malachite green in 2 per cent. acetic acid and then washed well. Unbleached (sulphite or sulphate) pulps appear deep green; thoroughly bleached fibres are unaffected; partly-bleached fibres assume intermediate shades (see also Grant, *Proc. Tech. Sect. Paper Makers' Assoc.*, 1935, **16**, 97).

(5) *Alexander's Stain* (*Paper*, 1934, **33**, 138).—The slide is stained for 1 minute with a solution of 0·2 g. of Congo red in 300 ml. of water. It is then dried (without washing) in air, and immersed for 1 minute in 3 drops of a solution of 100 g. of calcium nitrate in 50 ml. of water; 1 drop of Herzberg stain is added, and the mixed stains are allowed to act for three minutes. Coniferous fibres appear pink, and deciduous fibres blue.

With Korn's modification (Federal Specification, *loc. cit.*, and *Papier Fabr.*, 1925, **23**, 781) the fibres are floated in three drops of a solution of 100 g. of calcium nitrate in 25 ml. of water for 1 minute, and 1 drop of Herzberg's stain is then added.

The results are not always reliable for soft cooked, sulphate coniferous pulps. With this exception Korn's method, though simpler, is reliable.

(6) *Lofton–Merritt Stain* (Federal Specification, *loc. cit.*).—The fibres are stained for 2 minutes in a mixture containing 1 volume of a 2 per cent. solution of malachite green and 2 volumes of a 1 per cent. solution of basic fuchsin in water. The excess is blotted off, a few drops of 0·1 per cent. hydrochloric acid are added, and this too is blotted off and replaced by water. Unbleached sulphate fibres are stained blue or blue-green, and unbleached sulphite fibres purple. If tests on samples of known origin produce purple sulphate fibres or green sulphite fibres, more malachite green or fuchsin must be added, respectively (see also, Grant, *loc. cit.*).

(7) *Mitsumata and Gampi.*—These may be distinguished by means of 17·5 per cent. sodium hydroxide solution, as the former shows a bead-like structure due to swelling.

Textile Fibres

Preparation of the Sample.—The individual fibres in a sample of a textile may, as a rule, be distinguished easily under the microscope and separated, but if the fibres are dyed to a deep shade this is more difficult, and, moreover, the subsequent staining is affected. The fibres must therefore be decolorised, *e.g.* by means of boiling water or by cold or warm 1 per cent. acid or alkali; sodium hyposulphite ($Na_2S_2O_4$, known in the trade as "hydrosulphite") is very effective. Wool fibres, which hold the dye so tenaciously that they are attacked themselves before they are decolorised, may be treated with a warm mixture of 1 volume of 20 per cent. titanous chloride solution and 2 volumes of conc. hydrochloric acid.

Ignition in a small flame is frequently an aid to identification (*e.g.* casein, animal fibres and acetate rayon give distinctive odours and leave characteristic residues).

(1) ANIMAL FIBRES.—Millon's reagent stains animal fibres a pink colour in 2 minutes; vegetable fibres do not react. A 1 per cent. solution of picric acid produces a yellow colour with animal fibres, but will not stain vegetable fibres. This is a useful confirmatory test, especially for dyed fibres which are incompletely decolorised.

(2) CASEIN FIBRES are stained red by a saturated solution of benzo-purpurine in water (Bergen, *Amer. Dyestuff Rep.*, **1936, 25,** 146), and bright blue by a saturated solution of indigo carmine in very dilute sulphuric acid (Herzog, *Melliand Textilber.*, **1931, 12,** 768).

(3) CORDAGE FIBRES.—(*a*) *Swett's Test* (ANALYST, **1918, 43,** 227) distinguishes manila from all other "hard" rope fibres. The test requires considerable care, and experiments should be made on samples of known origin. It is also important that the alcohol and ammonia are not weakened by exposure to the air, and that prolonged exposure to ammonia fumes (which discharges the red colour) is avoided.

(*b*) *Stegmata Stains.*—The whole ash left on ignition is mounted in aniline oil or phenol; the stegmata or silicified cells of abacia (manila) are then visible as rows of rectangular hollow bodies. The ash of coconut fibres contains siliceous globules which are of various sizes, and have a foam-like inner structure; sisal, however, produces no siliceous skeletons, although characteristic elongated calcareous aggregates are visible. The action of 20 per cent. hydrochloric acid is to render the siliceous structures more readily visible; the formation of needles on addition of sulphuric acid indicates the presence of calcium.

Alternatively, the ash may be dropped into a 2 per cent. solution of potassium ferrocyanide and hydrochloric acid then added. The round stegmata of coconut usually turn blue, whilst a lighter blue is often shown by those of manila.

(*c*) *Jute Stain.*—The fibres are placed in 1 drop of a solution of 1 g. of phloroglucinol in 80 ml. of alcohol, and 1 drop of conc. hydrochloric acid is added. Unbleached jute fibres, which are highly lignified, turn dark violet-red.

(4) COTTON.—(*a*) *Cuprammonium Test* (Muller, *Faserforsch.*, **1929, 7,** 205).—The reagent is a solution of 2 g. of cupric hydroxide in 100 ml. of 25 per cent. ammonia, to which is added a few drops of ruthenium red; it should be kept in a dark bottle. When immersed in a few drops of the reagent cotton swells, forming light blue "balloons" which are separated by constrictions, the remnants of cuticle being pink; mercerised cotton, however, swells uniformly. The method can only be used qualitatively.

(*b*) *Hübner's Stain* (*J. Soc. Chem. Ind.*, **1908, 27,** 105; Lange, *Z. angew. Chem.*, **1903, 16,** 599.)—Mercerised cotton is stained deep blue or black by a 20 per cent. solution of iodine in a saturated solution of potassium iodide; unmercerised cotton is not stained. The method is good, provided that decolorisation has been thorough.

(*c*) *Fluorescence Analysis* sometimes serves to distinguish old and new cotton, since the latter has a violet fluorescence which turns to an ivory or brown colour as it ages. It is advisable to have authentic samples available for comparison. With mixtures the results should be assessed in conjunction with other evidence.

(5) FLAX, HEMP AND RAMIE.—(*a*) *Cross Sections* of the fibres are stained with a 1:10,000 solution of ruthenium red and washed. The cell-contents of flax and the middle lamellae of hemp are stained most prominently, and the respective lumens are circular and elongated.

(*b*) *Cuprammonium* (see above).—As flax swells, remnants of protoplasm are visible in the tortuous central tube, whilst the inner canal of hemp shows horizontal striations. The test is useless for highly-bleached fibres. Swelling may be arrested by transferring the fibre to a mixture of equal volumes of water, glycerin and alcohol.

(*c*) *Cyanin* (Herzog, *Melliand Textilber.*, **1932, 13,** 121, 181).—The fibres are macerated in a boiling 1 per cent. solution of sodium hydroxide and thoroughly

washed. They are then stained with a warm mixture of an almost saturated solution of cyanin, to which has been added 33 per cent. of glycerin, and are finally washed in a mixture of equal volumes of glycerin, water and alcohol and mounted in glycerin. Flax fibres remain colourless, but hemp is stained greenish-blue owing to lignification of the middle lamella.

(*d*) *Moisture Test* (Nodder, ANALYST, 1923, **48**, 88).—If a fibre is wetted and drawn between moist finger-tips, and then held vertically and viewed from above, flax moves in a clockwise direction; hemp sometimes does the same, but more usually it moves in the opposite direction, although less vigorously.

(*e*) *Potassium Dichromate* (Hanausek, *Z. Farben-Ind.*, 1908, **7**, 105).—Flax swells more readily than hemp in a solution of potassium dichromate in dilute sulphuric acid; the central canals are wavy and straight, respectively.

(*f*) *Ramie* (Molisch, *Akad. Wiss. Wien., Sitzungsber.*, 1920, (i), **129**, 261).—The fibres are ignited over a low flame, and the whole ash is mounted in aniline. Numerous characteristic spherical cystoliths with spicules are prominent. The cystoliths of manila are smaller, and characteristic rows of stegmata are apparent (see above); flax has neither cystoliths nor stegmata.

(6) RAYONS.—The results of Tests (*a*) to (*k*) are summarised in the Table. Italics indicate the respect in which any one particular rayon differs from all the others, and the corresponding test is therefore specially suitable for identification of such rayons. Special treatments (*e.g.* delustring and "ironproofing") and experimental rayons not commonly found on the market at present, may provide deviations from these results. Tests (*d*), (*e*) and (*f*) are the most satisfactory for quantitative work.

(*a*) *Acetone* (A.S.T.M., *Standards on Textile Fibres*, 1937, p. 36) is added to the fibres, which are observed under the microscope.

(*b*) *Diphenylamine* (0·3 g.) is dissolved in 20 ml. of conc. sulphuric acid and 10 ml. of glacial acetic acid (*ibid.*, pp. 33 and 36), and the reagent is added to the fibres on a slide.

(*c*) *Erie Fast Orange-CG* (a 0·2 per cent. solution) is allowed to stain the fibres for 3 minutes, and the fibres are then washed and examined. A rise in temperature during staining deepens the colour (*ibid.*, p. 36; Hahn, *Text. Col.*, 1931, **53**, 487).

(*d*) *Wright's Stain* (Johnson, *Text. World*, 1929, **75**, 49).—A suspension of methylene blue-2B in water is precipitated with about one-half the quantity of "eosin yellowish" (? Colour Index No. 768), and the mixture is stirred well. The precipitate is separated in a centrifuge and, subsequently, by decantation or filtration. A white or light-coloured sample of the dry fibre is covered with a cold solution of the stain* in alcohol, which is boiled for a few seconds. The fibres are then washed and examined. All rayons give a violet colour if wet before being stained.

(*e*) *Hahn's Picric Acid Test* (*Text. Col.*, 1931, **53**, 487).—The sample is immersed for 3 minutes in a cold or luke-warm solution containing 1 per cent. of picric acid and 0·2 per cent. of Soluble Blue-2B extra; it is then washed well.

(*f*) *Hahn's Eosin Test* (*loc. cit.*).—To a cold solution of 0·2 g. of Soluble Blue-2B extra, 0·1 g. of eosin and 1 g. of tannic acid in 100 ml. of hot water is added 0·2 ml. of 10 per cent. hydrochloric acid. The staining-period is 3 minutes, after which the fibres are washed.

(*g*) *Iodine* (0·015 g.) is dissolved in 1 litre of a 5 per cent. solution of potassium iodide and 2 drops of acetic acid are added (Kasche, *Melliand Textilber.*, 1932, **13**, 420). The fibres are immersed and then washed.

(*h*) *Dark-Field Illumination* (magnification, 350 diameters).—Large, brilliantly-reflecting particles, derived from delustrants, may occur in all rayons; however, they are not usually confused with the particles referred to in the Table.

(*i*) *Silver Nitrate* (Levey, *Chemist-Analyst*, 1936, **25**, 14).—To a solution

* *i.e.* presumably the above-mentioned precipitate.—J. G.

containing 4 g. of sodium thiosulphate is added a solution containing 1 g. of silver nitrate until the cloudiness disappears; a solution containing 4 g. of sodium hydroxide is then added, the mixture is diluted to 100 ml., and the final solution is cooled and filtered. Staining is carried out at the boiling-point.

(*j*) *Polarised Light.* See Table.

(*k*) *Cross-Sections* (A.S.T.M., *loc. cit.*; Rayon Committee, *Proc. Amer. Assoc. Text. Chem. Colorists*, 1934, **23**, 241).—See Table; photomicrographs are given in the references.

Test	Type of Rayon			
	Cellulose acetate	Viscose	Cuprammonium	Nitrocellulose
(*a*)	*Dissolves*	Unaltered	Unaltered	Unaltered
(*b*)	Colourless; dissolves	Colourless; dissolves	Colourless; dissolves	*Deep blue*; dissolves
(*c*)	Colourless or pale orange	Colourless or pale orange	*Deep orange*	Colourless or pale orange
(*d*)	Violet (partly disintegrated)	*Blue*	*Violet*	Deep blue
(*e*)	Yellow	Colourless	*Blue*	Colourless
(*f*)	Lavender	Lavender	*Blue*	Lavender
(*g*)	*Yellow*	Almost colourless	Almost colourless	Almost colourless
(*h*)	Almost transparent	*Innumerable very fine white particles*	Almost transparent	Almost transparent
(*i*)	Colourless	*Brown*	Colourless	Brown
(*j*)	*Silver gray*	Streaked	*Uniform* yellow or green	Streaked
(*k*)	Few indentations	*Serrated edges*	*Circular*	Few indentations

(7) Silk.—The precipitate formed by the addition of sodium hydroxide solution to a solution of 25 g. of crystalline nickel sulphate in 500 ml. of water, is washed and subsequently dissolved in a mixture of 125 ml. each of conc. ammonia and water. Ordinary silk fibres swell and dissolve in the cold, whilst "wild" (tussah) silk is unaffected, although it behaves similarly to ordinary silk on warming.

(8) Wool (Herzog, *Melliand Textilber.*, 1931, **12**, 768).—The fibres are stained in a saturated solution of indigo carmine in very dilute sulphuric acid, which is prepared in the cold; they are then mounted in conc. glycerin. Only the damaged portions of wool become bright blue. If the specimen is counter-stained with picric acid before being mounted, the undamaged parts become yellow and the damaged parts green. Damage caused by mechanical means, treatment with alkali or exposure to light is detectable in this way. J. G.

The International Standard for the Gonadotrophic Substance of Human Urine of Pregnancy: Chorionic Gonadotrophin

We have been asked by the Director of the Department of Biological Standards, National Institute for Medical Research, to publish the following statement:

At the Third International Conference on the Standardisation of Hormones, held at Geneva on August 11th to 13th, 1938, it was decided that international standards should be established for certain hormones of the anterior lobe of the pituitary gland and analogous substances found in urine and serum, and that international units should be defined in terms of a weight of each such standard.

It was further decided that the final preparation of these standards, their dispensing in a form suitable and convenient for the use of the laboratory worker, and their storage, preservation and subsequent distribution should be undertaken by the National Institute for Medical Research, Hampstead, London.

We are asked to announce that the preparation of the international standard for the gonadotrophic substance of human urine of pregnancy (chorionic gonadotrophin) has now been completed. The standard has been prepared from substantial amounts of material generously provided by six firms in different countries. The individual samples having been approved by the Conference, a mixture was made and the standard has been finally dispensed in the form of 10 milligram tablets and packed in a very convenient manner.

The international unit has been defined as the specific gonadotrophic activity of 0·1 milligram of the standard preparation, an amount of activity similar to that required, under the conditions used by many workers, to cause cornification of the vaginal epithelium of the immature rat. Each tablet contains approximately 100 international units.

As in the case of the international standards for other hormones, drugs and vitamins, the international standard for the gonadotrophic substance of human urine of pregnancy (chorionic gonadotrophin) is held, on behalf of the Health Organisation of the League of Nations, at the National Institute for Medical Research, Hampstead, London, and is distributed therefrom to national control centres established in other countries for local distribution to laboratories, institutes and research workers; and to workers in other countries in which the establishment of national control centres has not yet been completed.

With regard to the supply of the standard for chorionic gonadotrophin to those requiring it in the United Kingdom, application should be made to the Department of Biological Standards, National Institute for Medical Research, Hampstead, London, N.W.3.

British Standards Institution

THE following Standard Specifications have been issued*:

NO. 135—1939. BRITISH STANDARD SPECIFICATIONS FOR BENZOLES. (PURE BENZOLE, PURE BENZOLE FOR NITRATION, MOTOR BENZOLE, 90'S BENZOLE, INDUSTRIAL BENZOLE.)

These Specifications are based on the Standard Specifications Nos. 1, 2, 3, 4 and 5, 1938, respectively, of the National Benzole Association.

NO. 458—1939. BRITISH STANDARD SPECIFICATIONS FOR XYLOLES. (2° XYLOLE, 3° XYLOLE, AND 5° XYLOLE.)

These Specifications are based on the Standard Specifications Nos. 9, 10 and 11, 1938, respectively, of the National Benzole Association.

NO. 805—1939. BRITISH STANDARD SPECIFICATIONS FOR TOLUOLES. (PURE TOLUOLE, PURE TOLUOLE FOR NITRATION, 90'S TOLUOLE, 95'S TOLUOLE.)

These Specifications are based on the Standard Specifications Nos. 6, 7, 8A and 8B, 1938, respectively, of the National Benzole Association.

NO. 479—1939. BRITISH STANDARD SPECIFICATIONS FOR COAL TAR NAPHTHAS (COAL TAR SOLVENT NAPHTHAS 96/100 AND 90/160, COAL TAR HEAVY NAPHTHA 90/190, COAL TAR HEAVY NAPHTHAS [UNRECTIFIED] 90/190 AND 90/200.)

The methods of testing and descriptions of apparatus given for the preceding four Specifications are those recommended by the Standardisation of Tar Products Tests Committee and issued in their publication "STANDARD METHODS OF TESTING TAR AND ITS PRODUCTS."

NO. 830—1939. BRITISH STANDARD SPECIFICATION FOR WINCHESTER BOTTLES (OF 80 OZ. AND 90 OZ. NOMINAL CAPACITY).

The Specification refers to the most usual sizes of Winchester bottles, one of 80 fluid ounces nominal capacity to meet the requirements of those who need a bottle to contain safely half a gallon of liquid and a larger bottle suitable for liquids sold by weight, which will allow a reasonable expansion and vapour space. The nature of the liquid will determine the volume of that liquid for which the bottle is suitable. The Specification provides for these sizes of bottle, either plain or fluted, with four alternative methods of closure. Screw stoppers and caps and corks should be used with due regard to the nature of the liquid to be contained in the bottles fitted with

* Obtainable from the Publication Department British Standards Institution, 28 Victoria Street, London, S.W.1. Nos. 135, 458, 479, and 805, price 3s. 6d. net, post free 3s. 8d. Nos. 830, and 836, price 2s. net, post free 2s. 2d.

them. To facilitate storage and packing, the same diameter has been adopted for the two sizes of bottle, the two capacities being secured by a variation in the height.

To allow of small variations in shape resulting from differing methods of manufacture, the shapes of the bottles are not standardised in detail, and for similar reasons no requirements are laid down as to the minimum thickness of the glass. A minimum weight of glass, however, is specified (40 and 44 oz. for 80 and 90 fluid oz. bottles, respectively), and it should be the aim of manufacturers to secure a reasonably uniform distribution of the glass and to avoid abrupt variations in thickness.

No. 836—1939. British Standard Specification for Whale Oil.

This Specification forms part of a series of British Standards for Marine Animal and Fish Oils, the preparation of which was authorised by the Chemical Divisional Council.

The Specification is intended to include the technical provisions necessary for the supply of the material to which it refers, but does not purport to include all the necessary provisions of a contract.

It does not provide for all grades of good whale oil of merchantable quality, some oils being of higher and some of lower grade than that provided for in the Specification.

Whale oil is defined as the product obtained from various parts of the whale (excluding sperm oil). It must be free from contamination or admixture with other oils and fats, and shall answer to the following requirements:

Moisture and dirt.—Not more than 0·5 per cent. of moisture and/or dirt shall be present when determined by the methods described. An apparatus for the determination is described and illustrated.

Colour.—When the liquid-filtered oil is matched in a 1-in. cell with Lovibond glasses at 25° to 30° C., the red component of the glasses shall not exceed 6 units. In preparing oil for this test it is essential that the temperature shall not materially exceed 30° C.

Saponification value.—Not lower than 182 or higher than 205 when determined by the specified method.

Acidity.—The oil shall be free from mineral and added organic acids. Its acidity, when determined by the specified method, shall not exceed 6 per cent. of free fatty acids calculated as oleic acid.

Unsaponifiable matter.—The oil shall not contain more than 2·0 per cent. as determined by the Society of Public Analysts' method (Analyst, 1933, **58**, 203).

Sampling and size of sample.—Representative samples, each measuring not less than 400 ml., shall be taken, wherever possible in triplicate, from original containers or from the bulk, and shall be packed in clean, dry air-tight, non-absorbent containers, on which the sample has no action. The containers shall be of such a size that they are nearly filled by the sample.

Recommended methods for sampling fats and fatty oils are given in B.S. No. 627—1935.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Phosphorus in Fruits and Fruit Products. H. W. Gerritz. (*J. Assoc. Off. Agric. Chem.*, 1939, **22**, 131–137.)—Zinzadze's method has been adapted to the use of the neutral wedge photometer (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 130) and to the analysis of samples prepared by wet ashing. (1) *Zinzadze's reagent.*—The directions and precautions given by Zinzadze (*cf.* Analyst, 1932, **57**, 411; 1936, **61**, 198) are followed exactly, a ten-fold dilution being used in the actual determination. (2) *Preparation of sample.*—A portion of the sample containing 1 to 3 mg. of phosphorus pentoxide is transferred to a 500-ml. Kjeldahl flask. For the determination of phosphorus in the water-soluble portion of fruits and fruit juices 25 ml. (equivalent to 3·75 g.) of the sample solution prepared according to the official methods (*Methods of Analysis, A.O.A.C.*, 1935, XXVI, [*b*] or [*c*] are used, and for jams and jellies 50 ml. of the prepared solution. Fifteen ml. of conc. nitric acid, 5 ml. of conc. sulphuric acid, 5 or 6 small glass beads, and a few pieces of broken porcelain are added, and the liquid is boiled over a moderate flame until copious fumes of sulphuric acid are evolved. If there is

pronounced charring, the liquid is cooled slightly and a little more nitric acid is cautiously added (with jams 3 additions of the acid may be necessary). The liquid is boiled again until fumes are evolved, 0·5 ml. of 60 per cent. (reagent quality) perchloric acid is added, and the "fuming" is continued for a few minutes. The digest should now be colourless or slightly greenish-yellow. If necessary, a further 0·5 ml. of the perchloric acid is added and the "fuming" is continued for 3 or 4 minutes. The liquid is cooled somewhat, 50 ml. of water are added, and the solution is boiled until fumes appear, to remove traces of nitric acid, after which it is cooled, treated with 25 ml. of water, made up to 100 ml. and thoroughly mixed. (3) *Determination of phosphorus.*—Twenty ml. are transferred to a 100-ml. flask (a Kohlrausch sugar flask has been found convenient) with a mark at 60 ml. Three drops of sodium alizarin sulphonate solution (0·20 g. of sodium alizarin monosulphonate dissolved in 100 ml. of water and filtered) are added, and the liquid is neutralised with conc. potassium hydroxide solution (prepared from phosphate- and arsenate-free potassium hydroxide and stored in a paraffin-lined container); the colour of the liquid is adjusted to yellow by means of *N* potassium hydroxide solution and *N* sulphuric acid, each being added dropwise with constant mixing until a single drop of the sulphuric acid just changes the colour to yellow. The liquid is diluted to the 60-ml. mark and mixed. The flask is placed in a boiling water-bath and brought to that temperature, a drop or two of the *N* sulphuric acid being added if the colour becomes pink. Exactly 10 ml. of Zinzadze's reagent (10-fold dilution) are added, the liquid is mixed, and the heating in the bath is continued for exactly 20 minutes. (It is important that the standards and unknown solutions be heated at the same temperature.) The solution is cooled, diluted and mixed, and the colour intensity is measured by means of the neutral wedge photometer, with a 1-inch cell and No. 66 filter (4·5 MM Corning dark pyrometer red No. 241; with B & L "Smoke C" glass, wedge filter 65, *i.e.* the same as 66 plus a half MM of Jena BG18, is used) and Jena 0–2 neutral wedge. The method covers a range up to 0·6 mg. of phosphorus pentoxide in the final 100 ml. of solution. Standards covering this range are prepared by placing 0, 2, 4, 6, 8, 10 and 12 ml. of standard phosphate solution containing 0·05 mg. per ml. (prepared by dissolving 0·1917 g. of pure dry potassium dihydrogen phosphate in about 200 ml. of water, adding 10 ml. of *N* sulphuric acid and 6 drops of 0·1 *N* potassium permanganate solution, and diluting to 2 litres) in 100-ml. flasks with marks at 60 ml. Two ml. of dilute (1 : 1) sulphuric acid and 3 drops of the indicator are introduced into each flask, with water to make about 20 ml. These standards are then treated in the same way as the sample, beginning with the neutralisation, developing the colour, cooling, making up to volume, and determining the colour intensity in the neutral wedge photometer. If alkali of suitable purity has been used, the 0 standard should give a reading not more than 10 to 15 mm. greater than the reading with pure water. A large-scale graph of the standards is made, mg. of phosphorus pentoxide being plotted against photometer readings. The sample photometer readings are converted into mg. of phosphorus present in the final 100-ml. portion by means of this graph. The photometer need be calibrated only once for each batch of reagents if the adjustment is not altered. In the analysis of heterogeneous samples, *e.g.* fresh fruit, for total phosphorus, it may be necessary to digest a larger

portion in order to eliminate sampling and weighing errors. It is then convenient to take double the sample (7·5 g.) and add double the volume of sulphuric acid (10 ml.). The digest is made up to 200 ml. and a 20-ml. aliquot part is transferred to a 100-ml. flask for colour development. The weight of sample digested may be varied to suit the nature of the sample, provided that the final 20-ml. portion contains about 1 ml. of sulphuric acid and 0·2 to 0·6 mg. of phosphorus pentoxide. The method has been found to be applicable to the analysis of materials of higher phosphorus-content, such as semolina macaroni. Zinzadze mentions that iron, nitrate and arsenic interfere with the development of the colour with his reagent. Nitrates are not present in solutions prepared as described, and neither iron nor arsenic is ordinarily present in sufficient quantity in fruit or fruit products to cause interference. If fruit were contaminated with spray residue to the extent of, say, 0·1 grain of arsenic trioxide per lb., it would make a positive error in the phosphate determined of only 1·4 mg. per 100 g. If the presence of excessive quantities of iron or arsenic is suspected the procedure is modified as follows:—The above directions are followed to the point "until a single drop of the sulphuric acid just changes the colour to yellow." Ten ml. of exactly *N* sulphuric acid and 10 ml. of 8 per cent. sodium bisulphite solution are added, and the solution is diluted to 60 ml. The flask is heated in a boiling water-bath for 1 hour and the directions given above are then followed, beginning "Exactly 10 ml. of Zinzadze's reagent (10-fold dilution) are added." The standards must, of course, then be treated in the same manner (see also Abstract, p. 455). E. M. P.

Contamination of Parsley with Arsenic. J. M. Rowson and C. E. Waterhouse. (*Pharm. J.*, 1939, **142**, 329.)—The arsenic-content of a sample of dried parsley (leaf and a little stalk) was 6 p.p.m. That of the herb before drying was: leaf and stalk, 1; root (with a little adhering earth), 1·5 p.p.m. (washing the fresh herb very thoroughly before analysis did not alter this result), and for a similar sample from a different locality it was 3 p.p.m. As fresh parsley normally loses from 60 to 80 per cent. of its weight on drying, the arsenic must have been present in the original sample. Cultivation experiments are in progress to investigate the possibility of the assimilation of arsenic by parsley. E. B. D.

Unusual Adulterant of Olive Oil (Quinoline Yellow). J. Pritzker and R. Jungkunz. (*Z. Unters. Lebensm.*, 1939, **77**, 254–256.)—A specimen of olive oil described as "guaranteed pure and genuine extra vierge" gave constants differing little from the normal values for genuine olive oil, but when examined in ultra-violet light the sample showed an intense grass-green fluorescence. Parallel determinations were therefore made with two samples of olive oil of French origin and a sample of tea-seed oil of Chinese origin. The "guaranteed pure" oil showed great similarity to pure olive oil and to tea-seed oil and, since it did not answer to tests for arachis, sesame, cotton-seed or kapok oil, suspicion was aroused that it was a mixture of olive and tea-seed oils. The possibility of its consisting entirely of tea-seed oil was excluded by the m.p. of the phytosteryl acetate prepared from it, *viz.* 120° C., the m.p. of phytosteryl acetate prepared from tea-seed oil being 156 to 157° C. In the fractional precipitation of its fatty acids (Kreis and Roth, *Z. Unters. Nahr. Genussm.*, 1913, **25**, 84) the sample yielded

only 0·68 g. of solid fatty acids from 20 g. of oil, whereas pure olive oil should yield at least 2·2 g. Another suspicious feature was the fact that the lead salts began to separate in 4 hours, as with tea-seed oil, whereas with pure olive oil the separation begins within an hour. These results pointed to the presence of a considerable amount of another oil, possibly tea-seed oil. In addition, the oil contained an artificial dye which was not detected by the usual methods (*Schweiz. Lebensmittelbuch*, 4th Ed., Bern, Zimmermann & Co., 1937), but was detectable by the method of Lecoq and Prince (*Guide pratique d'Analyses alimentaires*, Paris: Vicot Frères, 1921). The oil (5 g.) was dissolved in 5 ml. of petroleum spirit, the solution was shaken with 5 ml. of glacial acetic acid, and the acid layer was separated and evaporated to dryness. A deep yellow residue was obtained. It was not possible to identify the dye by dyeing tests, but when the fluorescence of the oil in ultra-violet light was compared with that of a series of dyes dissolved in almost colourless arachis oil, the conclusion was reached that the dye was quinoline-yellow. A. O. J.

Water Melon Seed Oil. A. J. Nolte and H. W. von Loesecke. (*J. Amer. Chem. Soc.*, 1939, **61**, 889–891.)—Air-dried seeds of melons (culls) grown in south central Florida, of the variety Cuban Queen (*Citrullus vulgaris*), a round or slightly oval melon with brownish black seeds, had the following composition:—moisture, 8·84; fat (ether extract), 26·52; protein, 17·31; ash, 2·36 (water-soluble, 0·05; insoluble, 2·31); undetermined, 44·97 per cent. The yellowish-brown oil was extracted with petroleum spirit and filtered; it contained 0·06 per cent. of volatile constituents and had the following characteristics:—sp.gr. at 25/25° C., 0·9197; n_D^{20}, 1·4669; saponification value, 197·4; iodine value (Hanus), 133·8; Reichert–Meissl value, 0·29; Polenske value, 0·72; acetyl value, 7·5; Hehner value, 89·2; acid value, 0·42; unsaponifiable matter, 1·19 per cent.; unsaturated acids (corr.), 78·96 per cent., with iodine value, 166·6; saturated acids (corr.), 14·56 per cent. The fatty acids consisted of palmitic, 8·84; stearic, 5·61; arachidic, 0·72; oleic, 13·03; linolic, 68·38 per cent. D. G. H.

Seed Fat of *Hodgsonia capniocarpa*. T. P. Hilditch, M. L. Meara and W. H. Pedelty. (*J. Soc. Chem. Ind.*, 1939, **58**, 26–29.)—The seed kernels of this plant, which is a Malayan climber of the Natural Order *Cucurbitaceae*, yielded to petroleum spirit 62 per cent. of a pale yellow oil with saponification equivalent 284·3, iodine value 65·5, acid value 0·8, and unsaponifiable matter 0·3 per cent. The component acids were found to consist of myristic, 0·7; palmitic, 39·5; stearic, 8·3; arachidic, 0·7; hexadecenoic, 1·0; oleic, 26·0; linolic, 23·8 per cent. mol. The chief constituents of the fat were found to be: "oleo"dipalmitins, 33; "oleo"-palmitostearins, 27; palmito-di"oleins," 24; tri-unsaturated glycerides (oleo-linolins), 13 per cent. mol., with possibly very small quantities of stearo-di"oleins." A small proportion (2·5 per cent.) of fully-saturated components was present. The occurrence of 13 per cent. of tri-unsaturated glycerides is distinctive, owing to the fact that oleic and linolic acids are both present in about the same high proportion. A comparison of the fatty acids with the component acids of shea butter, in which the ratio of unsaturated to saturated acids is very similar, but in which linolic acid forms only a very minor proportion of the unsaturated group,

gave:—fully saturated (*a*) shea butter, 4·5; (*b*) *H. capniocarpa*, 2·5; mono-unsaturated, (*a*) 34·5, (*b*) 60; di-unsaturated mono-saturated, (*a*) 56·5; (*b*), 24; and tri-unsaturated, (*a*) 4·5; (*b*), 13 per cent. The specific nature of the glycerides in this fat is evidently the result of combinations of four major component acids (palmitic, stearic, oleic, and linolic) to form triglycerides, whereas in fats previously studied it has been necessary to consider only the three acids, palmitic, stearic and oleic. D. G. H.

Chemical Examination of *Bragantia wallichii* (Lour). B. L. Manjunath and M. S. Shankara Rao. (*J. India Chem. Soc.*, 1938, **15**, 646–648.)—The root of *Bragantia wallichii*, Lour. (Sanskrit Chakrani, N. O. *Aristolochiaceae*, growing in the Western Ghats) has been used in the proportion of 4 g. of root made into a paste with 14 ml. of lemon juice, with beneficial results, in cases of cholera and diarrhoea. Fifty g. of powdered root were successively extracted with solvents and yielded extracts as follows: petroleum spirit, 0·48; ether, 0·78; chloroform, 0·49; ethyl acetate, 0·48; absolute alcohol, 3·70 per cent. Constituents volatile in steam amounted to 0·05 per cent., and 0·03 per cent. of alkaloid was present. About 50 kg. of root were extracted, and the extracts were examined in detail. The fatty acids of the dark brown oil from the petroleum spirit extract were palmitic, a small amount of crude lignoceric, oleic and linolic. A yellow substance separated from the ethereal extract, and the ethereal solution was successively extracted with 5 per cent. hydrochloric acid, potassium bicarbonate, potassium carbonate and potassium hydroxide solutions. The hydrochloric acid extract was combined with the hydrochloric acid extract of the chloroform extract, and these gave positive tests with reagents for alkaloids. The yellow substance, which was also obtained on acidifying the alkali extracts from ether and chloroform, and from the ethyl acetate extract, was identified as *iso*aristolochic acid after crystallisation from glacial acetic acid and alcohol. Unlike the acid itself, the methyl derivative was tasteless. D. G. H.

Action of Strychnine and Quinine on Bordeaux B. D. B. Dott. (*Pharm. J.*, 1939, **142**, 328–329.)—As previously stated (*Pharm. J.*, 1938, **141**, 261; Abst., ANALYST, 1938, **63**, 827) the strychnine separated from compound syrup of glycerophosphates is yellow even after re-precipitation with ferrocyanide, owing to the formation of a compound of strychnine with the acid of Bordeaux B. Also, the strychnine cannot be correctly determined by titration. It may be crystallised from the impure precipitate by extraction of a benzene solution with water and acid followed by addition of alkali and extraction with chloroform and evaporation, this process being repeated if necessary. When solutions containing equivalent quantities of strychnine hydrochloride and the dyestuff (termed sodium azorubrate) are mixed, part of the strychnine is precipitated as an azorubrate and part remains in the filtrate, which is coloured red. Analysis by two methods (*a*) and (*b*), showed that the precipitate contained (*a*) 52·06 and (*b*) 52·31 per cent. of strychnine. Theoretically, the di- and mono-strychnine salts contain 59·43 and 42·22 per cent. respectively of alkaloid. With quinine hydrochloride solution the dye gave a precipitate containing 57·0 per cent. of quinine (theoretical for di-quinine salt, 58·69 per cent.). It is considered therefore that Bordeaux B

should not be used for colouring liquids containing strychnine or other precipitatable alkaloids. Solubilities in water at room temperature:—strychnine azorubrate, 1 : 1000; quinine azorubrate 1 : 2270. E. B. D.

Valerian : A New Alkaloid. J. J. Blackie and D. Ritchie. (*Pharm. J.*, 1939, **142**, 299–300.)—A water-soluble base possessing physiological activity is present in appreciable quantity in dried valerian root. The base is soluble in cold or hot water and in alcohol, very sparingly soluble in ethyl acetate, and insoluble in acetone, chloroform or ether. It is precipitated by ether in flocculent particles from its solution in amyl alcohol. It has not yet been found possible to crystallise the base or to prepare crystalline derivatives. When distilled in a high vacuum (0·01 mm.) it decomposes at 200° C. Apparently it is not a pyrrole derivative, the presence of which in the root was reported by Cionga (*Comptes rend.*, 1935, **200**, 780), as the vapours evolved on heating the base with zinc dust did not give the characteristic red colour on pine wood. Intravenous injection of the base into a cat under urethane anaesthesia caused a fall in blood pressure, with a secondary effect upon the heart; it also had an inhibitory effect on peristalsis. These physiological effects require confirmation when the base has been obtained in a pure condition. Methods of separating the base from the root by extraction with amyl alcohol after removal of oil, resins, etc., are described in detail. E. M. P.

Methyl Alcohol Content of Tobacco Leaf and Ribs. W. Preiss. (*Z. Unters. Lebensm.*, 1939, **77**, 272–281.)—It has been shown by numerous workers that tobacco smoke contains methyl alcohol derived mainly from methyl pectic esters, and it is generally agreed that the methyl alcohol yield from unfermented tobaccos may reach 0·9 per cent., and that during fermentation that of cigar tobacco is reduced to 0·04—0·2 per cent. and that of cigarette tobacco to about 0·5 per cent., so that, as a rule, the pale tobaccos yield more methyl alcohol than the dark tobaccos. When the coarser parts of the leaf (midrib and large veins) are used, Neumann-Wender (*Münch. Med. Woch.*, 1933, **80**, 737) observed an increased yield of methyl alcohol, constituting a potential source of danger to the smoker. Since the pectic esters are largely responsible for the methyl alcohol occurring in the smoke, it appears necessary to determine the amounts of pectin and lignin and the methyl alcohol derived from them. Also, a determination of methyl alcohol derived from lignin would indicate the proportion of woody tissue in the tobacco. Methods based upon those of von Fellenberg (*Biochem. Z.*, 1918, **85**, 45, 118) were used to distinguish between the methyl alcohol derived from these two sources. To determine the methyl alcohol derived from pectin, 1 g. of finely powdered tobacco was treated with 20 ml. of water and allowed to stand for 2 hours. The mixture was treated with 5 ml. of 10 per cent. sodium hydroxide solution, allowed to stand overnight, and after the addition of 2·5 ml. of 20 per cent. sulphuric acid, it was distilled until 18 to 19 ml. had been collected. This distillate, after the addition of 1 ml. of 20 per cent. sulphuric acid, was redistilled until 14 to 15 ml. of distillate had been collected. To this distillate 5 drops of 10 per cent. sodium hydroxide solution and 5 drops of 10 per cent. silver nitrate solution were added, and 12 ml. of distillate were collected. Finally, this distillate was redistilled from animal charcoal until exactly 10 ml. had been collected. An

aliquot portion (3 ml.) was taken for the determination of methyl alcohol. For the determination of the total methyl alcohol from pectin and lignin, 0·5 g. of powdered tobacco was mixed with 15 ml. of 72 per cent. sulphuric acid and boiled gently in a distillation apparatus at such a rate that not more than 1 or 2 ml. of distillate were collected in 10 minutes. When the liquid had cooled, 25 ml. of water were added, and 27 to 28 ml. were distilled. The distillate was neutralised to litmus paper with 10 per cent. sodium hydroxide solution and, after the addition of 5 drops in excess and 5 drops of 10 per cent. silver nitrate solution, the mixture was distilled, and 20 to 21 ml. of distillate were collected. The treatment with sodium hydroxide and silver nitrate was repeated, and the distillate of 12 ml. was redistilled from animal charcoal until exactly 10 ml. of distillate were obtained; an aliquot portion (3 ml.) of this was used for the determination of methyl alcohol. The methyl alcohol concentrates (3 ml.) obtained by these methods were treated in 100-ml. flasks with 1 ml. of alcoholic sulphuric acid (20 ml. of absolute alcohol and 40 ml. of conc. sulphuric acid diluted with water to 200 ml.) and 1 ml. of 5 per cent. potassium permanganate solution, shaken well, allowed to stand for exactly 2 minutes, and treated with 1 ml. of 8 per cent. oxalic acid solution and 1 ml. of conc. sulphuric acid. As soon as the solution became colourless, 5 ml. of fuchsin-sulphurous acid solution (5 g. of fuchsin, 12 g. of crys. sodium sulphite and 100 ml. of *N* sulphuric acid diluted to 1 litre) were added. The solution was allowed to stand for exactly one hour, water was added (25 ml. for amounts up to 1 mg. of methyl alcohol in 10 ml. of distillate, 50 ml. for larger amounts), and the solution was compared in a colorimeter with standard solutions prepared from pure methyl alcohol treated in the same manner. The amount of methyl alcohol present was determined by means of a calibration curve constructed for the instrument. The Lange photo-electric colorimeter is recommended. The methyl alcohol derived from pectin bodies is very small in the large-leaved tobaccos of the alkaline group; in European pipe-tobacco it may reach about 0·4 per cent. Tobacco ribs yield less pectin methyl alcohol than the whole or stripped leaf. In oriental cigarette tobaccos of the acid group the pectin-methyl alcohol content is higher than in the alkaline group and amounts to about 0·5 per cent. Methyl alcohol derived from lignin amounts to about 0·2 per cent. in the large-leaved tobaccos, 0·4 per cent. and more in the ribs, and 0·12 per cent. in the stripped leaves. Oriental tobaccos yield about 0·1 per cent. of methyl alcohol from lignin. The amount of lignin methyl alcohol obtained is a measure of the lignified tissue in the tobacco product and may be used to determine approximately the amount of rib and vein present. The methods described provide a useful means of following the effects of the processes of tobacco manufacture upon its pectin and lignin contents.

A. O. J.

Biochemical

Estimation of Sulphanilamide in Biological Fluids. A. E. A. Werner. (*Lancet*, 1939, **1**, 18–20.)—When a solution of sulphanilamide is treated with *p*-dimethylaminobenzaldehyde a deep yellow colour is produced, or, if sufficient sulphanilamide is present, an orange precipitate. The colour of the yellow solution is remarkably stable, is proportional to the concentration of sulphanilamide, and is

sensitive to about 1 part in 500,000. It develops rapidly in solutions diluted 1 in 100 or 1 in 200, at which dilutions the normal constituents of urine do not interfere.

Determination in urine.—The urine is diluted with distilled water, so that the solution contains 0·5 to 1·5 mg. of sulphanilamide per 100 ml. To 9 ml. of this solution is added 1 ml. of a 3 per cent. solution of *p*-dimethylaminobenzaldehyde in 7 per cent. sulphuric acid. The yellow colour develops immediately and is compared directly with the colours developed by standard solutions under the same conditions. Sulphanilamide is partly eliminated from the body as the acetyl derivative, and to determine the amount of the drug present in this form, 1 ml. of the urine is heated with 2 ml. of *N* hydrochloric acid for 30 minutes in a boiling water-bath, cooled, neutralised with 2 ml. of *N* sodium hydroxide solution and diluted to 10 ml. This solution is suitably diluted, and 9 ml. are treated with 1 ml. of the *p*-dimethylaminobenzaldehyde reagent to determine the amount of total sulphanilamide; the difference between the free and total amounts gives the amount present as acetylsulphanilamide.

Estimation in blood.—One ml. of oxalated blood is added drop by drop from a pipette to 4 ml. of a 5 per cent. solution of trichloroacetic acid, and the resulting suspension is filtered. Two ml. of the filtrate are transferred to a graduated cylinder and diluted to 3·5 ml. with 0·25 *N* sodium hydroxide solution, and 0·5 ml. of the *p*-dimethylaminobenzaldehyde reagent is added. The colour is compared with the colours of standard solutions as before. To estimate total sulphanilamide, 2 ml. of the acid filtrate are heated in boiling water for 30 minutes and then neutralised and treated with the reagent. For accurate work a colorimeter can be used, as the intensity of the colour is strictly proportional to the concentration for the range 0·25 to 1·5 mg. per 100 ml. For approximate work a simple comparator can be used. Quantitative recoveries were obtained of sulphanilamide and acetyl sulphanilamide added to urine, and of sulphanilamide added to blood. There appeared to be some adsorption of acetylsulphanilamide, however, by the proteins precipitated from blood, but the adsorption was reduced to a minimum by adding 0·5 ml. of blood to 4·5 ml. of trichloroacetic acid solution. Two of the most important derivatives of sulphanilamide, namely, M. & B. 693 (2-sulphanilylaminopyridine) and Uleron (4′-dimethylaminosulphamido-4-aminobenzenesulphonamide) can also be estimated with the same degree of accuracy and sensitivity by this method. F. A. R.

Photometric Determination of Selenium in Tissues and Faeces. R. A. Gortner and H. B. Lewis. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 198–200.)—Horn's modification of the codeine sulphate reaction has been applied. The dry fat-extracted sample is digested in a Kjeldahl flask by heating with sulphuric acid (40 ml.) and yellow mercuric oxide (0·2 g.) until clear. The liquid is diluted to 50 ml. with conc. sulphuric acid, centrifuged for 15 to 20 minutes at 1800 r.p.m., and to 10 ml. are added 3 drops of saturated aqueous codeine sulphate solution. The mixture is kept for 7 hours in the dark, and the intensity of blue colour developed is determined photometrically by means of a Zeiss-Pulfrich photometer fitted with a yellow filter (S.57), which has been calibrated with solutions containing known amounts of selenium treated in a similar manner. Amounts of selenium down to 0·1 mg. were accurately determined. S. G. C.

Direct Colorimetric Method for the Estimation of Inulin in Blood and Urine. A. S. Alving, J. Rubin and B. F. Miller. (*J. Biol. Chem.*, 1939, **127**, 609–616.)—Inulin gives an intense, clear blue colour with alcoholic diphenylamine in hot acid solution, and this reaction has been made the basis of a method of estimating inulin. Since, however, fructose, and to a small extent glucose, give similar colours with the reagent, for accurate determinations these sugars must be removed by fermentation. A suspension of washed yeast cells (0·5 or 1 ml., respectively) is added to 5 or 10 ml. of plasma or serum in a 15-ml. centrifuge tube, which is then shaken, incubated at 38° C. for 30 minutes and centrifuged at high speed for 15 minutes. The supernatant plasma is treated with 8 volumes of acid cadmium sulphate reagent (13·0 g. of pure hydrated cadmium sulphate and 63·5 ml. of exactly *N* sulphuric acid made up to 1 litre with water) followed by 1 volume of 1·1 *N* sodium hydroxide solution. The mixture is shaken, allowed to stand for 15 minutes and filtered, and the filtrate is diluted, if necessary, so as to contain 2 to 12γ of inulin per ml. Five ml. of diluted urine (which usually does not need to be fermented) or of the filtrate from plasma or serum are transferred to a thick-walled test-tube (20 × 110 mm.), and 10 ml. of diphenylamine reagent are added. (The reagent is made by mixing 12 volumes of a 10 per cent. solution of pure diphenylamine in redistilled absolute alcohol with 192 volumes of a mixture of 80 volumes of conc. hydrochloric acid and 112 volumes of redistilled absolute alcohol. The solution does not keep for more than a week.) The tubes are tightly stoppered, immersed in a boiling water-bath for exactly 60 minutes and then plunged into cold water. The colour of the resulting solution is measured in a photometer. The Evelyn photo-electric colorimeter with a No. 660 filter proved most satisfactory, but the Zeiss–Pulfrich "Stufenphotometer" and the Dubosq colorimeter have also been used. A blank determination with distilled water is made at the same time, and the instrument is calibrated by measuring the colour given by solutions of inulin of known strength. The method was used to estimate the glomerular filtration rate, and a correction had to be applied for the small amount of colour produced by blood and urine containing no inulin. Inulin added to plasma or serum in amounts varying from 4 to 15 mg. per 100 ml. was determined with an error of 2 per cent. or less, and equally accurate results were obtained with plasma and urine from patients suffering from severe uremia or severe jaundice and with urines containing large quantities of protein. Good results were also obtained with the plasma and urine from diabetic patients, but for these fluids the fermentation technique had invariably to be employed. F. A. R.

Studies on the Nature of the Iodine in Blood. V. Trevorrow. (*J. Biol. Chem.*, 1939, **127**, 737–750.)—The iodine-contents of numerous fractions prepared from whole blood or blood plasma were estimated by the method of Trevorrow and Fashena (*J. Biol. Chem.*, 1935, **110**, 29; 1936, **114**, 351; Abst., ANALYST, 1935, **60**, 628). It was found that all the iodine present in blood could be extracted with boiling alcohol or cold acetone; added potassium iodide and thyroxine were extracted quantitatively with either solvent, but the iodine of added thyroid gland could not be extracted with alcohol. It was concluded from these experiments that blood iodine is not present in protein combination. Attempts to remove

iodine from the plasma proteins by ultra-filtration were to a large extent unsuccessful, for only 10 to 20 per cent. of the iodine was present in the filtrate. It was found, however, that thyroxine added to the plasma also did not pass through collodion membrane, but that added potassium iodide did; this suggested that not more than 20 per cent. of the plasma iodine is in inorganic combination. This conclusion was confirmed by the observation that most of the iodine was precipitated with the proteins on heating with acetic acid or on treatment with zinc sulphate and sodium hydroxide solutions, and that added thyroxine (but not added potassium iodide) was likewise precipitated. It therefore appears that the iodine in blood is present in the form of a compound closely resembling thyroxine, and this hypothesis is supported by the fact that all the iodine in blood can be extracted with butyl alcohol. However, whilst thyroxine remains wholly in the butyl alcohol layer when this is shaken with sodium hydroxide solution, the iodine from blood is partitioned between the two phases. Di-iodotyrosine, on the other hand, is completely extracted by alkali. The exact nature of the iodine-containing compounds in blood has not yet been established with certainty. F. A. R.

Purification and Some Properties of Renin. O. M. Helmer and I. H. Page. (*J. Biol. Chem.*, 1939, **127**, 757–763.)—An active extract of the pressor substance of pig kidney cortex was prepared by extraction of the acetone-dried gland with sodium chloride solution. This solution was acidified to *p*H 4·5 and, after removal of the inert proteins, the active principle was precipitated by adding sodium phosphate solution to the filtrate. The precipitate was extracted with sodium chloride solution acidified with acetic acid, and the filtrate was dialysed and freed from inert protein by adding alkali to *p*H 6·5. The resulting product contained 0·4 mg. of nitrogen per ml. It has strong pressor activity, as had also a picrate and a reineckate prepared from it. The active principle was precipitated by ammonium sulphate at 40 per cent. concentration and by sodium chloride at full concentration, both at *p*H 3 to 4. The solution was inactivated by heating it above 56° C. Positive reactions were obtained for guanidine groups and for pentose, but a test for adrenine was negative. The most active fraction prepared caused a rise of 30 mm. in the arterial blood pressure of a dog when injected in quantities representing 0·027 mg. of nitrogen per kg. of body-weight. In cats, 0·009 mg. of renin nitrogen produced a rise of 32 mm. The evidence so far available suggests that the pressor substance is not adrenine. F. A. R.

Colorimetric Reaction for the Quantitative Estimation of Nicotinic Acid. E. Bandier and J. Hald. (*Biochem. J.*, 1939, **33**, 264–271.)—Nicotinic acid, metol (*p*-methylaminophenol sulphate) and cyanogen bromide in aqueous solution give a yellow solution, the colour of which is stable and proportional to the amount of nicotinic acid present. A measured amount (up to 9 ml.) of the aqueous solution to be tested, containing 0·005 to 0·25 mg. of nicotinic acid, is run into a graduated 20-ml. flask, which is then heated for 5 minutes on a water-bath at 75° to 80° C. One ml. of a freshly-prepared 4 per cent. aqueous cyanogen bromide solution is added, and the mixture is placed on the water-bath for another 5 minutes and then cooled to room temperature. (A 5 per cent. solution of cyanogen bromide can readily be made by adding potassium cyanide to a saturated

aqueous solution of bromine until the latter is just decolorised.) Ten ml. of a freshly-prepared solution (about 5 per cent.) of metol are added, and the solution is made up to 20 ml. with water. After the mixture has stood for 1 hour at room temperature excluded from light, the intensity of the colour is measured in a Pulfrich photometer (filter S43) with a blank solution containing cyanogen bromide and metol in the other cell. When the amount of nicotinic acid taken is in excess of 0·25 mg., it is futile to dilute the final coloured solution; the original solution must be diluted and the determination repeated. A calibration curve with known amounts of nicotinic acid is constructed. Nicotinamide also gives a yellow colour, but this is considerably stronger than with nicotinic acid, so that when the amide and the acid occur together, it is better to hydrolyse the solution before carrying out the estimation. Nicotinic acid can be determined in biological material, for example in yeast, by the following procedure:—Ten ml. of 2 *N* sodium hydroxide solution are run into a 20-ml. graduated flask, 5 g. of dry yeast are added, and the neck of the flask is closed with a wad of non-absorbent cotton-wool. The flask is shaken, placed on a boiling water-bath for 30 minutes and allowed to cool, and 1·8 ml. of conc. (36 per cent.) hydrochloric acid are introduced, drop by drop, with shaking. The solution, which now has a *p*H of 4 to 6, is diluted to 20 ml. with water. The contents are thoroughly mixed, a portion is centrifuged, exactly 1 ml. (equivalent to 250 mg. of yeast) is transferred to a second centrifuge tube, and 9 ml. of acetone are slowly added from a burette. The tube is closed with a rubber stopper, shaken for a few minutes and centrifuged, whereby a very small (about 0·3 ml.) aqueous layer and a nearly colourless layer of aqueous acetone are formed. Three ml. of the acetone layer (equivalent to 75 mg. of yeast) are mixed with 3 ml. of water in a round-bottomed flask, and the acetone is removed at room temperature by means of a water vacuum-pump. The residual aqueous solution is quantitatively transferred with the aid of *N*/15 potassium acid phosphate solution to a 20 ml. graduated flask. The rest of the procedure is the same as that described above. Quantitative recoveries of added nicotinic acid were obtained by this method. F. A. R.

Determination of Cholesterol by Chromic Oxidation. F. Kayser and C. Mathieu. (*Bull. Soc. Chim.*, 1939, **6**, 715–717.)—The method depends on the complete oxidation of the digitonin-cholesterol complex by means of potassium dichromate in presence of silver nitrate as catalyst. The recommended procedure is as follows:—The digitonin-cholesterol precipitate (about 1 g.) is collected on a Jena glass filter and the filter is transferred to a flask carrying a ground reflux column. Twenty ml. of 0·05 *N* chromic mixture (500 ml. of 0·1 *N* aqueous potassium dichromate solution with sulphuric acid to 1 litre) and 0·2 ml. of 25 per cent. silver nitrate solution are added. The liquid is heated on an oil-bath and kept at the boiling-point for 40 minutes. After cooling, a little water is added, and the liquid is transferred to a 200-ml. graduated flask, treated with 0·4 ml. of 5 per cent. sodium chloride solution and made up to 200 ml. An aliquot portion is filtered through a Jena glass filter, and 20 ml. of the filtrate are treated with 20 ml. of water (the analysis must be carried out in a normal acid solution) and a crystal of potassium iodide, and titrated with 0·02 *N* sodium thiosulphate solution,

starch being used as an indicator. The complete oxidation of 1 mg. of cholesterol requires 0·3938 ml. of *N* dichromate solution, and the oxidation of the digitonin-cholesterol complex requires 0·254 ml. E. M. P.

Potentiometric Titration of Carotenoids with Gold Trichloride Solution. P. Karrer and W. Jaeger. (*Helv. Chim. Acta*, 1939, **22**, 314–322.)—In a previous paper (*cf.* ANALYST, 1938, **63**, 835) attention was directed to the error introduced by the presence of carotenoids in the estimation of tocopherol by titration with gold chloride solution. The oxidation of several carotenoids by this reagent has now been studied quantitatively. The carotenoids were dissolved in a small amount of ether, and the solution was diluted with 5 to 10 times its volume of alcohol and titrated with 0·01 *N* aqueous gold chloride solution at 65° to 75° C., the time required varying from 140 to 345 minutes. It was found that α- and β-carotene, lycopene, xanthophyll and zeaxanthin required 8 equivalents of gold chloride. Astacene and rhodoxanthin required 2 equivalents. Crocetin, bixin, fucoxanthin and violaxanthin were not oxidised. F. A. R.

Conversion of Carotene into Vitamin A_2 by some Fresh-water Fishes. R. A. Morton and R. H. Creed. (*Biochem. J.*, 1939, **33**, 318–324.)—Perch (*Perca fluviatilis*) were successfully maintained in captivity on a diet of blow-fly larvae, and these fish were used to determine whether carotene is a provitamin A and provitamin A_2. The fish were divided into two groups, one receiving blow-fly larvae only, the other being given the larvae coated with powdered carotene. After two months the fish were killed, and the amounts of vitamins A and A_2 present in various organs were determined by spectroscopic examination of the blue solution formed by adding antimony trichloride solution to the unsaponifiable fraction. The vitamin A content of the livers of the fish that had received carotene was twice as high as that of the control group and the amount of vitamin A_2 was three times as great. Even so, however, the storage levels of the fish receiving carotene was not raised to that of larger fish feeding naturally, and it would appear that, whilst carotene is both a provitamin A and a provitamin A_2, it is not the only provitamin. The amount of carotene in zooplankton, such as constitute the diet of perch, was found to be relatively small. Dace also were fed on blow-fly larvae with and without added carotene, and estimations of the vitamin contents of various organs indicated a substantial increase in the amount of both vitamins as a result of adding carotene to the diet. F. A. R.

Distribution of Vitamins A and A_2. II. J. A. Lovern, R. A. Morton and J. Ireland. (*Biochem. J.*, 1939, **33**, 325–329.)—In continuation of the work previously described (*cf.* ANALYST, 1938, **63**, 358) other species of fish have now been examined. A large sturgeon (*Acipenser sturio*) yielded 1·47 kg. of liver oil containing 1 per cent. of vitamin A, and part of the intestines gave 29·3 g. of oil containing 10 per cent. of vitamin A. The liver contained 30 g. of vitamin A esters and 7 g. of vitamin A_2 esters, and the intestines 6 g. and 0·5 g. of vitamin A and vitamin A_2 esters respectively. The ratio vitamin A/A_2 was much higher in the intestines than in the liver, as was also found with salmon, trout and halibut. Lamperns (*Petromyzon fluviatilis*) contained little vitamin A, although it was

present at a number of sites, and even less vitamin A_2. Dogfish (*Squalus acanthias*) gave a liver-oil poor in vitamin A, and the intestines also contained only a small amount. It is suggested that the dogfish and some other species do not utilise vitamin A in fat absorption to the same extent, for instance, as the halibut, cod and salmon. The herring-gull (*Larus argentatus*), the skua (*Megalistris catarrhactis*) and the gannet (*Sula bassana*) were found to contain more vitamin A in their intestines than such animals as the rabbit; no vitamin A_2 was detected in any of the organs of these sea-birds. The one gannet that was examined contained quite large amounts of vitamin A in the lungs.

A preliminary study was also made of the provitamin D contents of sterols from different sources. Cholesterol from cod-liver oil, salmon-liver oil and similar sources contained 0·03 to 0·1 per cent. of provitamin D, but the sterol from halibut stomach contained about 0·5 per cent. The sterol from halibut spleen showed a broad absorption band at 275 $m\mu$ due to an unknown substance that was absent from the spleen sterols of bullock (0·02 per cent. of provitamin D) and sheep (0·06 per cent.). F. A. R.

Distribution of Vitamins A and A_2. III. J. A. Lovern and R. A. Morton. (*Biochem. J.*, 1939, **33**, 330–337.)—Preliminary experiments were made to determine the distribution of vitamins A and A_2 in the different layers of halibut intestine. From the lumen to the outer wall, these layers are as follows:—(1) *Mucosa*, (*a*) *epithelium*, (*b*) *tunica propria*, (*c*) *stratum compactum* and (*d*) *stratum granulosum*. (2) *Muscularis* (*a*) *circularis and* (*b*) *longitudinalis*. (3) *Serosa* (*a*) *serosa* proper and (*b*) *sub-serosa*. Two methods were investigated. In the first, a hand-cut section, 1 to 2 mm. thick, of the fresh intestine was cleared by immersion in a homogeneous mixture of glucose and golden syrup and a photomicrograph was made with a "Pointolite" bulb as illuminant. A second photomicrograph was then made with a sheet of Wood's glass placed between the light source and the microscope (with, of course, a longer exposure), so that only ultra-violet light was utilised. Comparison of the two photographs showed that the mucosal coats were much more opaque to ultra-violet light than to visible light, whilst the muscle layers showed little sign of such selective absorption.

A better method of investigating the distribution of the vitamins in the different layers is to separate them mechanically, and to estimate the vitamin-contents in each layer by the usual procedure. The *tunica propria* was found to be far richer in vitamin A than the other mucosal coats, whilst these in turn were much richer than the muscle and *serosa*. The distribution of fat was similar to that of the vitamin A. The mechanism of fat absorption in fishes is different from that obtaining in mammals, proceeding directly through the mucosal epithelium and not by a lymphatic system. The *stratum compactum* forms a barrier to the transport of fat as droplets which accumulate in the adjacent *tunica propria*, and the hypothesis is advanced that vitamin A esters in combination with protein may be instrumental in transporting these droplets from the *tunica propria*. F. A. R.

Halibut Intestinal Oil. J. A. Lovern, T. H. Mead and R. A. Morton. (*Biochem. J.*, 1939, **33**, 338–343.)—A sample of the oil from fresh halibut intestine was subjected to molecular distillation, Hickman's constant-yield oil (*J. Ind. Eng.*

Chem., 1937, **29**, 1107) being used. It was thus shown to contain 95 per cent. of its vitamin A in the form of esters. Oil from halibut intestines that were a few days old was found to contain a high proportion of free fatty acid, and 20 to 30 per cent. of the vitamin existed in the free state. The presence of the fatty acids facilitated cyclisation of the vitamin. Oil from autolysed halibut intestines contained about 25 per cent. of material that was not glyceride, vitamin ester or sterol ester, and a fraction soluble in water that exhibited selective absorption at 261 $m\mu$. The elimination curve of vitamin A_2 closely resembled that of vitamin A.

F. A. R.

Occurrence of Vitamin B_2 (Lactoflavin). II. Vitamin B_2 in Animal Organs. J. Schormüller. (*Z. Unters. Lebensm.*, 1939, **77**, 346–357.)—In continuation of the investigation of the occurrence of vitamin B_2 (*Z. Unters. Lebensm.*, 1939, **77**, 1; Abst., ANALYST, 1939, **64**, 215), the lactoflavin-content of different animal organs has been determined by the methods previously described. The results for the organs of recently killed cattle (γ per 100 g. of dry material) were as follows:—liver, 5550 to 7410; kidney, 6170; lung, 2220; heart, 1750; spleen, 1090. Corresponding figures for the organs of the edible mussel were:—liver, 1763; heart, 645. In order to investigate the degree of combination of the lactoflavin with the colloidal carrier (yellow enzyme) the flavin bodies were extracted with solvents and separated into their high-molecular and low-molecular components by dialysis in the manner previously described (*loc. cit.*). By extraction of ox-liver with cold water, it was found that 88·1 per cent. of the extracted lactoflavin is combined with the colloidal carrier. By extraction of pulped liver with water at 37° C., 31·3 per cent. of the total flavin present was extracted, and this figure was increased to 41·07 per cent. when the liver was ground after preliminary treatment with liquid air. By extraction of liver with various solvents it was shown that the amount of flavin extracted varied with the solvent used, the greatest amount being obtained by the use of anhydrous glycerin. By incubation of pulped liver with water at 37° C. and periodic analysis of the extracted flavins it was shown that no measurable destruction of the vitamin occurred, but that it was separated from the colloidal carrier in progressively increasing amounts. It therefore appears that there is no danger of loss of the vitamin by autolysis during the storage of the liver. Investigation of the flavin-content of different parts of the kidney of the horse showed that the lactoflavin-content varied in parts of the same organ which are structurally and functionally different. Similar determinations of the lactoflavin-content of different portions of the heart of the horse showed that, as in muscle, an inverse relationship exists between the lactoflavin-content and the glycogen-content. Other physiological relationships are discussed.

A. O. J.

Separation of Lactoflavin and Lactoflavin Phosphate. A. Emmerie. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 290–292.)—Lactoflavin can be extracted from aqueous solution by benzyl alcohol and other aromatic alcohols, whereas only traces of lactoflavin phosphate are thereby extracted. Thus 76 per cent. of the lactoflavin was found to be present in the benzyl alcohol phase when equal volumes of the alcohol and water (pH 5 to 7) were shaken and allowed to separate.

The determination of lactoflavin and lactoflavin phosphate was made by measuring the intensity of the colour in a Zeiss-Pulfrich photometer. The procedure recommended for determining the amount of free and combined flavin in urine is to prepare an extract as previously described (*Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, 1936, **6**, 136), and evaporate the eluate from the lead sulphide to dryness under reduced pressure. The residue is taken up in water, and the solution is oxidised with potassium permanganate and hydrogen peroxide in acetic acid solution, and then neutralised and shaken with benzyl alcohol. The difference between the intensity of the colour of the aqueous extract before and after benzyl alcohol extraction is equivalent to the amount of lactoflavin present, whilst the colour of the aqueous extract after the extraction is due to lactoflavin phosphate. F. A. R.

Colorimetric Estimation of Tocopherol (Vitamin E). II. Adsorption Experiments. A. Emmerie and C. Engel. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 283–289.)—Tocopherol can be separated from carotenoids and vitamin A by chromatographing a benzene solution (5 ml.) on a column (30 × 12 mm.) of "Floridin XS" earth, which has been purified by digestion with hot conc. hydrochloric acid, and washed with water, alcohol and benzene. The chromatogram is developed with 25 ml. of benzene, which is sufficient to wash the tocopherol quantitatively through the column into the filtrate. The carotenoids and vitamin A are retained on the adsorbent. Wheat-germ oil is freed from carotenoids and rendered quite colourless by this treatment. Alumina retains tocopherol as well as carotenoids and vitamin A from petroleum spirit, and the tocopherol is quantitatively eluted by methyl alcohol. A vitamin A concentrate treated with the ferric chloride—dipyridyl reagent used for estimating tocopherol (*cf.* ANALYST, 1939, **64**, 216), slowly loses its activity as a result of oxidation by the reagent. The reaction takes much longer, however, to reach the end-point both with vitamin A and with carotene than with tocopherol. F. A. R.

Bacteriological

The Methylene Blue Reduction Test and the Keeping Quality of Milk. C. H. Chalmers. (*J. Dairy Res.*, 1938, **9**, 351–355; *Bull. Hyg.*, 1939, **14**, 333.)—In view of the poor correlation of the methylene blue test with the keeping quality of milk under conditions prescribed in Memorandum No. 139 of the Ministry of Health (Special Designations) Order, 1936, the author carried out a series of tests with milk, holding it for varying periods of time at 15° C. before submitting it to the test. The milk was produced at 7.0 a.m., cooled at the farm, collected at 10.0 a.m. and taken immediately to the laboratory. Two hundred samples were examined, and each was divided into six portions which were held at 15° C. for periods of time ranging from 1 hour to 23 hours. The portions examined after 1 hour at 15° C. showed very poor correspondence between reduction time and keeping quality, and the correspondence was little better after 7 hours. The portions kept for 16 hours at 15° C. showed better, though not very good, correlation, whereas with those held for 23 hours the correlation was fairly close. The

author suggests that during the time in which the milk is kept before being tested the species of organisms change progressively, so that the predominant type at any one time differs from that at any other. D. R. W.

Water

Quantity of Zinc in Sea Water. G. Bertrand. (*Bull. Soc. Chim.*, 1939, **6**, 697–700.)—The following method was used for the determination of zinc in water from the Channel or the Atlantic Ocean:—One litre of sea water was evaporated to a quarter of its volume in a porcelain dish after addition of 1 ml. of pure conc. hydrochloric acid. A slight excess of 20 per cent. barium chloride solution (about 35 ml.) was added gradually to precipitate the sulphates, and the liquid was treated, without filtering, with about 10 drops of perhydrol, 40 ml. of pure ammonia, free from carbonate, and then gradually with pure lime to displace the magnesium. With water from the Mediterranean, which has a higher salt-content, the volume was reduced to a third after addition of 2 ml. of hydrochloric acid, and 50 ml. of ammonia were used. The precipitate was separated by centrifuging, and the solution was transferred to a conical flask. The basic constituents of the precipitate were dissolved in the minimum quantity of pure hydrochloric acid, the liquid was diluted to about 250 or 300 ml., and the precipitation with ammonia and a little lime was repeated. After centrifuging, the liquid was added to the first one. For greater accuracy a second and, if necessary, a third treatment with acid and alkali was made. The liquid finally obtained was analysed by the method of Bertrand, Bertrand and Javillier, and Bertrand and Mokragnatz (*Comptes rend.*, 1892, **115**, 939, 1028; *Bull. Soc. Chim.*, 1906, **1**, 63; 1923, **33**, 1539), the zinc being weighed as the anhydrous sulphate. With water from the Dead Sea, 250 ml. of the water diluted with an equal volume of pure water were taken for the analysis. The following results were obtained:

Sea water	Volume of water litres	Zinc sulphate mg.	Zinc, mg.	Zinc, g. per cubic metre
North Sea ..	2	15·3	6·197	3·10
Channel	1	8·5	3·442	3·44
Channel	3	27·0	10·935	3·65
Atlantic Ocean ..	2	15·2	6·156	3·08
Mediterranean ..	2	18·7	7·573	3·79
Dead Sea ..	0·25	2·7	1·094	4·37

E. M. P.

Organic

Rapid Method of Determining Water in Butyl Alcohol. G. Lazzari. (*Chim. e Ind.*, 1939, **21**, 68–69.)—The method depends on the turbidity produced when butyl alcohol containing water is treated with a mixture of 2 volumes of pure benzene and 1 volume of pure acetone (rectified at 56° C.). The recommended procedure is as follows :—Twenty ml. of the butyl alcohol under examination and 2 ml. of the benzene-acetone mixture are pipetted into a 50-ml. flask of colourless glass with a tightly fitting stopper. Water is added dropwise from a burette

graduated in twentieths of a ml., and after each addition the flask is stoppered and shaken vigorously for a few seconds. The reading is taken when the liquid shows a persistent opalescence. Too vigorous shaking appears to destroy the emulsion, possibly by raising the temperature, but if the liquid is allowed to stand at the temperature of the experiment the turbidity reappears. The addition of 0·025 ml. of water in excess gives a definite opalescence, so that the end-point can be accurately determined. By using a micro-burette and observing the turbidity in a nephelometer, very accurate results can be obtained. For ordinary work, such as the determination of water in butyl alcohol which is to be used for dehydrating organic substances, it may be of assistance to use a device such as observing in diffuse daylight writing placed 15 cm. behind the flask and taking as the end-point the point at which the writing cannot be deciphered. The water-content, when the temperature is 16° C., can be calculated from the equation

$$\text{Volume per cent. of water} = -4{\cdot}27n + 15{\cdot}8,$$

where n is the volume of water added, in ml. For temperatures not far removed from 16° C., the relation is

$$\text{Volume per cent. of water} = -4{\cdot}27n + 15{\cdot}8 + 0{\cdot}07(t - 16),$$

where t is the temperature. E. M. P.

Behenic Acid in the Seed Oil of *Pongamia glabra*, Vent. B. L. Manjunath and M. S. Shankara Rao. (*J. Indian Chem. Soc.*, 1938, **15**, 653.)—The crude oil obtained from the seeds of *Pongamia glabra* (Hongey in Kannada, Karanja and Naktamala in Sanskrit and Sukchain in Hindi) gradually forms a solid deposit after standing for several days. This deposit was repeatedly washed with methyl alcohol to remove resins, and after all the karanjin had been extracted (Beal and Katti, *Zentrbl.*, 1926, **2**, 596; Limaye, *Proc. Indian Sci. Congress*, 1925, **1**, 18), the fatty acids in the residue were liberated and their methyl esters were distilled under reduced pressure (0·5 mm.). From the first of the two chief fractions (b.p. 199–205° C. at 0·5 mm.) behenic acid (m.p. 78°–79° C.) was liberated and purified by repeated crystallisation from methyl alcohol. The free acid of the second fraction (b.p. 207–212° C. at 0·5 mm.) and residue appeared to consist of lignoceric acid. Since behenic acid was not found by Sudborough *et al.* (*J. Indian Inst. Sci.*, 1923, **6**, 93) in the refined oil, it is probably removed during the refining process. D. G. H.

Oil of *Aleurites trisperma*. E. D. G. Frahm and D. R. Koolhaas. (*Rec. Trav. Chim. Pays-Bas*, 1939 **58**, 277–282.)—The oil from an authentic specimen of *Aleurites trisperma* had the following constants: sp. gr. at 15·5°/15·5° C., 0·9344; n_D^{25}, 1·4980; acid value, 4·9; saponification value, 190·8; Reichert-Meissl value, 0·61; hexabromide value, 0; iodine value (Wijs), 133·1; iodine value (Rosenmund-Kuhnhenn), 127·6; diene number (Ellis and Jones), 43·2; unsaponifiable matter, 0·5 per cent.; total fatty acids, 93·1 per cent.; saturated fatty acids (Bertram), 17·0 per cent. From the diene number it was computed that the oil contained 47·3 per cent. of elaeostearic acid, but this amount was not sufficient to account for the iodine value found, and it was suspected that an unsaturated acid other than elaeostearic and oleic acids was present. A portion of the fatty acids

was therefore treated with ozone, and the oxidation product was steam-distilled. The volatile acids were fractionated and shown to consist of *n*-valeric and caproic acids, identified as anilides, and pelargonic acid, identified as its zinc salt. Azelaic acid was identified in the non-volatile portion. Oxidation of the fatty acids with alkaline potassium permanganate solution similarly gave a mixture, in which *n*-valeric acid, caproic acid, pelargonic acid and 9 : 10-dihydroxystearic acid were identified. The presence of pelargonic acid among the oxidation products suggested that 9:12-linolic acid was present in the original oil, a suspicion that was confirmed by the identification of sativic acid (m.p. 174° C.) among the products obtained by more cautious oxidation with an excess of potassium permanganate amounting to not more than 10 per cent. of the amount calculated from the diene number and iodine value. This method has also been used to detect linolic acid in other oils, *e.g.* the oil of *Parinarium corymbosum*. Further calculations, based on the iodine value of linolic acid, indicated that the oil contained 18·0 per cent. of linolic acid and 10·8 per cent. of oleic acid, and the mean molecular weight of the saturated acids indicated that the amounts of palmitic acid and stearic acid present (only these could be detected) were 9·1 per cent. and 7·9 per cent. respectively. Thus the composition of the oil of *Aleurites trisperma* may be represented as: elaeostearin, 49·4; linolin, 18·8; olein, 11·3; palmitin, 9·5; stearin, 8·3; unsaponifiable matter, 0·5 per cent. These values differ widely from those previously reported by Jamieson and McKinney (*Oil and Soap*, 1935, **12**, 146). F. A. R.

Drying Oil of *Licania crassifolia* Benth. M. Sesseler and P. A. Rowaan. (*Chem. Weekblad*, 1939, **36**, 208–209.)—In the course of investigations into a substitute for Chinese wood oil, experiments were made with oils related to oiticica oil (from the seeds of *Licania rigida* Benth.) and obtained from the seeds of the plants *L. heteromorpha* Benth. ("japoballi") and *L. macrophylla* Klotzsch ("sponsehoedoe") which occur in Surinam (Dutch West Indies). These yielded only 0·5 to 1·0 per cent. of an oil soluble in petroleum spirit, and are considered of little interest. The seeds of *L. crassifolia* Benth. ("kwepilan"), however, gave a yield of 5 per cent. of oil by the expression method and 25 per cent. by extraction with petroleum spirit. They are pear-shaped, and have an average length of 10 mm. and an average thickness at the thickest part of 5 mm., and the endosperm contains about 60 per cent. of oil. Analytical data for the expressed and extracted oils, respectively, were as follows:—sp.gr. (15°/15° C.), 0·9588, 0·9555; n_D^{20}, 1·5367, 1·5292; acid value, 1·3, 2·9; saponification value, 191·5, 189·2; unsaponifiable matter, 1·3, 1·4 per cent.; saturated acids (Bertram), —, 7·1 per cent.; iodine value (Wijs, 1 hour), 163·2, 164·7; thiocyanogen value (24 hours), —, 118·8; diene value, 69·5, 71·0; carbonyl value (Leithe, *Fette Seifen*, 1938, **45**, 615), —, 105·0. The mean mol. equiv. of the fatty acids was 278, and the acids contained 18-C-atoms. The "heating-test" (*Proc. Amer. Soc. Testing Materials*, 1935, **35**, Part 1, 1397) showed that the oil formed a hard jelly after 9 minutes at 293° C., compared with 10·75 minutes for a "standard sample American tung oil" (maximum permissible value, 12 minutes). Analytical data for the separated fatty acids were:—iodine value (Wijs), 177·0; thiocyanogen value, 90·8; diene value, 63·2; hexabromide test, negative; these are in line with the gelatinising properties of the

oil. The unsaturated acids were investigated (after C. P. A. Kappelmeier, *Fettchem. Umschau*, 1935, **42**, 145) by saponification of the oil for 1 hour with a solution of sodium hydroxide in alcohol, followed by removal of the alcohol by distillation and precipitation of the fatty acids from a solution of the soap in water. The mixture was then extracted with benzene, and the extract was dried with anhydrous sodium sulphate, evaporated to a small volume, and left to crystallise in sunlight. After recrystallisation from acetone the m.p. of the resulting crystals was 91° C., but it was not possible to prepare a satisfactory specimen of the semicarbazone owing to the insolubility and sticky nature of the specimen. Extraction of the acids with ether or petroleum spirit, however (*cf.* Brown and Farmer, ANALYST, 1935, **60**, 570), and evaporation of the dehydrated extract in a vacuum, gave crystals (m.p. 75° C.), which were less sticky; whilst the use of carbon tetrachloride (*cf.* Steger and Van Loon, *Rec. Trav. Chim. Pays Bas*, 1938, **57**, 6) gave a dark red-brown solution containing a sticky residue, which deposited crystals with m.p. 95° C. The m.p. of the corresponding semicarbazone was 137° C., and the acid value was 188·4 (theoretical value for licanic acid, 191·7). The remainder of the sticky residue was recrystallised from petroleum spirit and then had m.p. 94° to 98° C. It is concluded that the glycerides of the oil are derived from the following acids:—licanic, approx. 60; linolic, approx. 20; oleic, approx. 5; saturated acids, 7 per cent. J. G.

Antioxygens in Natural Fats. IV. The Proportions and Properties of Antioxygenic Compounds in Various Extracted Seed Cakes. T. P. Hilditch and S. Paul. (*J. Soc. Chem. Ind.*, 1939, **58**, 21–24.)—The antioxygenic activities and other properties of palm-kernel, linseed, soya-bean, arachis and cotton-seed meals have been examined. The meals were prepared as described previously (*J. Soc. Chem. Ind.*, 1937, **56**, 23T; Abst., ANALYST, 1937, **62**, 206). The final yields of acetone-soluble concentrates were of the same order as before, establishing the same order for all the meals. The times in the oxidation tests, in hours, for the meals were, respectively:—palm-kernel, 5; linseed, 5; soya-bean, 14; arachis, 12; cotton-seed, 27. In each instance a small proportion of nitrogen was present in the concentrate; but, although there is a slightly closer parallelism between the nitrogen-contents and antioxygenic activities than between the degree of unsaturation and antioxygenic activities, it is not well marked. Almost complete suppression of activity occurs when an acetone solution of the original concentrate is exposed to a few bubbles of dry hydrogen chloride gas. With regard to the chemical nature of the natural antioxygenic compounds in seed meals, it is established that they show marked reducing power, which suggests the presence of reducing carbohydrates; ferric chloride solution gives a light brown colour; they contain 0·4 to 1·0 per cent. of combined phosphorus and 1 to 2 per cent. of combined nitrogen, and their antioxygenic properties disappear on prolonged heating with water or immediately on exposure to traces of hydrogen chloride; with the latter these properties are partly restored on subsequent careful neutralisation of the acid. The authors believe that they have established the fact that seeds contain natural antioxygenic compounds characterised by their basic or pseudo-basic nature. Although the identity of the basic component was

not determinable, there is some reason to think that basic oxygen, rather than basic nitrogen, may be the responsible factor for retardation of atmospheric oxidation. D. G. H.

Lignin and Related Compounds. The Ethanolysis of Spruce, Maple and other Woods. L. Brickman, J. J. Pyle, J. L. McCarthy and H. Hibbert. (*J. Amer. Chem. Soc.*, 1939, **61**, 868–869.)—Water-soluble ethanolysis products are isolated from various woods in higher yields and in purer condition than by the method previously described (Cramer, Hunter and Hibbert, *J. Amer. Chem. Soc.*, 1939, **61**, 523). In the new method the final extraction is made with benzene in an atmosphere of carbon dioxide. The wood meal (40-mesh) is air-dried and extracted for 48 hours with a 1:1 mixture of absolute ethanol and benzene and then for 24 hours with absolute ethanol, after which it is washed with hot running water for 12 hours, and dried first in the air and afterwards in a vacuum oven (20 mm. pressure) for 48 hours. For the ethanolysis the wood meal (1000 g.) is heated under reflux for 48 hours with dry ethanol (8 litres) containing anhydrous hydrogen chloride (160 g.) in an atmosphere of carbon dioxide. The mixture is cooled and filtered, and the residual meal is washed with hot ethanol. The combined ethanol extracts are concentrated under reduced pressure at 50° C. and dropped in a very fine stream into water which is being vigorously stirred. The precipitated ethanol lignin is filtered off and well washed. The aqueous solution and washings are concentrated, and the solution is extracted for 48 hours with benzene. The precipitated ethanol lignin is shaken five times with benzene for at least 2 hours each time to remove adsorbed oils, and the benzene extract is treated separately. The oils isolated from each portion are fractionated separately. In typical determinations the total oils extracted (expressed as per cent. on the weight of Klason lignin in the original material) were as follows:—spruce, (1) 10·9; (2) 14·2; maple, (1) 33·5, (2) 35·4; Douglas fir, 14·6; redwood, 13·9; red oak, 50·9; bamboo, 50·1; jute, 74·0; maize stalks, 59·6; rye straw, 40·6. The preliminary results indicate that the ethanolysis method is broadly suitable for isolating in high yields the constructional units from the lignin of a wide variety of plant types. D. G. H.

Combination of Formaldehyde with Collagen. J. H. Highberger and C. E. Retzsch. (*J. Amer. Leather Chem. Assoc.*, 1939, **34**, 131–148.)—The study of the combination of formaldehyde with collagen has been seriously hampered in the past by the lack of a method for the accurate determination of the amount of formaldehyde present in the tanned leather. The authors' method (ANALYST, 1938, **63**, 678) has, therefore, been used for this purpose, the experimental material being 2·0 g. of a purified collagen powder prepared from fresh, dry, ash-free steer hide (*cf. J. Amer. Leather Chem. Assoc.*, 1936, **31**, 93). This was tanned in 100 ml. of a 0·1 *N* solution of a phosphate buffer, to which were added sufficient 0·1 *N* sodium hydroxide solution to bring it to a desired *p*H (*i.e.* equivalent to 4 to 11 after the tanning process), and sufficient of a standardised 10 per cent. solution of formaldehyde to bring the concentration of this substance to 0·25 to 3·0 g. per 100 ml. The experiments were made within 1 or 2 hours of the preparation of the mixture in order to eliminate any reduction in the true concentration

of the formaldehyde owing to the occurrence of the Cannizzaro reaction; this is particularly important when the alkaline range is used. The tanning-period was normally 24 hours, and during this time the mixtures were agitated continuously in a shaking-machine. After the tanning the *p*H was determined by means of the glass electrode, and the tanned powders were washed over-night under standardised conditions with 15 to 19 litres of distilled water. A modification of the Schiff test (sensitiveness, 1:10,000) was then used to confirm the absence of free formaldehyde in the wash water, and the amount of formaldehyde in the tanned powder was finally determined by the authors' method (*loc. cit.*). Curves show the number of mg.-mols. of formaldehyde fixed per g. of collagen plotted against the tanning-time (up to 24 hours), the concentration of formaldehyde, and the *p*H, and the following conclusions are drawn:—When the concentration of formaldehyde is 1 per cent. or less, the reaction is confined to the free amino and guanidino groups provided by lysine and arginine, respectively, and one mol. of the formaldehyde reacts with each amino or guanidino group. Within this concentration range only the amino groups of lysine react at *p*H values of 8 or less, but above *p*H 8 the guanidino groups of arginine are involved. The data showing the effects of the tanning-time on the final *p*H indicate that a *p*H-equilibrium is established by the end of the first hour, although the fixation of formaldehyde continues to increase markedly throughout the first 8 hours. This suggests that the reaction is not ionic but molecular in nature, since it proceeds more slowly than would be expected of an ionic reaction. This is confirmed by the other results, which indicate that only un-ionised basic groups participate in the reaction. It follows that the function of the alkaline reaction in promoting formaldehyde tannage is to repress the ionisation of the basic groups. When the concentration of formaldehyde exceeds 1 per cent. it is fixed in larger quantities, and it is suggested that this may be due either to the extension of the reaction to the imino groups of the protein "back-bone," or to the reaction of the groups mentioned above with polymerised molecules of formaldehyde, or to both causes. This work fails to confirm the analogy which some workers (*e.g.* Küntzel, *angew. Chem.*, **1937**, **50**, 308) have drawn between urea-formaldehyde resins and formaldehyde-tanned leather. It is, however, not impossible that compounds of the type assumed by such a theory (*e.g.* dimethylol urea) may be produced as the result of the prolonged ageing of leather which has been tanned in high concentrations of formaldehyde; however, the present data have no bearing on this point. J. G.

Inorganic

Tetraphenylarsonium Chloride as an Analytical Reagent. H. H. Willard and G. M. Smith. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 186–188.)—The tetraphenylarsonium ion is likely to have uses as a reagent owing to its ability to form (*a*) insoluble salts by combination with ions such as perrhenate, permanganate, periodate and perchlorate, (*b*) insoluble complex mercuric, stannic, cadmium and zinc compounds, and (*c*) insoluble complexes with thiocyanate compounds of cobalt and iron. Applications of the reagent are to be given in subsequent papers; the present one is confined to the determination of the tetra-

phenylarsonium ion by potentiometric titration with iodine. The reaction involves combination of the ion with iodine and iodide: $(C_6H_5)_4As^{\bullet}+I_2+I' \rightarrow (C_6H_5)_4AsI_3$, the product, a rusty-orange precipitate, being insoluble in saturated sodium chloride solution. The potentiometric standardisation of tetraphenylarsonium chloride reagent solution is carried out as follows: Five to 10 ml. of the 0·01 to 0·03 *M* reagent solution are diluted to about 100 ml.; the reference and indicator electrodes are immersed in the liquid, which is titrated slowly, with constant stirring, with 0·02 *N* iodine solution containing 6 to 8 g. of potassium iodide per litre. During the titration the potential of the system decreases to a minimum; at the end-point there is a sudden increase in potential amounting to 25 to 35 millivolts per 0·01 ml. of 0·02 *N* iodine solution. Near the end-point the solution must be completely saturated with sodium chloride before the titration is completed. A smooth platinum wire and a calomel half-cell were used as indicator- and reference-electrodes respectively. The solution must be neutral or slightly acid. The alkalis and alkaline earths, nickel, cobalt(ous), chromium, manganese, borate, bicarbonate, acetate, phosphate, sulphate, citrate and tartrate, even in fairly high concentration, do not interfere. Nitrate in limited amount is allowable in neutral solution. S. G. C.

Modification of Bettendorff's Arsenic Test. W. B. King and F. E. Brown. (*J. Amer. Chem. Soc.*, 1939, **61**, 968–969.)—It has already been shown (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 168) that traces of mercuric chloride hasten the reduction of arsenic compounds by stannous chloride, and that the time required for the appearance and development of the arsenic suspension is a function of the concentration of mercuric chloride in the solution. The present work shows that the modified Bettendorff's test is catalytic and not induced. One hundred ml. of a mercurous chloride solution of 9×10^{-7} molar strength were saturated with chlorine gas and left overnight; the excess of chlorine was then swept out completely by a current of hydrogen chloride gas. Two hours were required for the greenish-yellow colour to disappear. Another sample of 100 ml. was saturated with hydrogen chloride gas; and the oxidised and non-oxidised solutions were compared as regards their catalytic effects by noting the length of time required for a changing suspension to become darker than an unchanging standard. Mercurous and mercuric chloride solutions were found to have equal catalytic powers, and it is concluded that the actual catalyst is the mercury atom formed by reduction of the salts. D. G. H.

Interaction of Zinc Salts with Alkalis. A. I. Nikurasin. (*J. Ob. Chemie.*, 1938, **8**, 1463–1464.)—The precipitation of zinc hydroxide with alkalis from solutions of the chloride and sulphate has been investigated potentiometrically, hydrogen, glass, zinc amalgam and mercuric oxide electrodes being used. For complex systems the *p*H is measured in separate samples, as the composition of the system changes during potentiometric titration; hence the conditions are near those of equilibrium and the investigation is made with a constant volume of solution. The titration curves show that a basic salt, having one molecule of zinc salt to 3 molecules of zinc hydroxide, is first formed; this reacts with more alkali to form zinc hydroxide. Hysteresis, which is shown by the difference in the direct and

reverse titration curves, is due to the slowness of the second stage. With a mercuric oxide electrode, the titration curves indicate the presence of acid and neutral zincate in solution at room temperature; the existence of the latter is confirmed by the curve with the zinc electrode. The curve for the titration of conc. sodium hydroxide with zinc chloride supports the hypothesis that sodium zincate, Na_2ZnO_2, first reacts with zinc chloride to precipitate zinc hydroxide and then slowly with the precipitate to form the difficultly soluble acid zincate. E. B. D.

Reaction of Tantalum, Niobium (Columbium) and Vanadium with Iodine. F. Korösy. (*J. Amer. Chem. Soc.*, 1939, **61**, 838–843.)—Tantalum, niobium and vanadium react with iodine vapour at dull red heat. Tantalum pentaiodide is stable, at least up to 500° C., but niobium and vanadium pentaiodides easily dissociate at their sublimation temperatures (about 400° C.). Incandescent tantalum wire will reduce tantalum pentaiodide in the vapour phase. The lower tantalum iodides, intermediate between the pentaiodide and metallic tantalum, may be obtained in nearly any desired average composition, but only one deep green compound can be extracted therefrom with water. The green ion was found to be charged positively, and the product is regarded as probably an analogue of Chapin's green chloride and bromide (*J. Amer. Chem. Soc.*, 1910, **32**, 323). A lower iodide of niobium was found to be stable *in vacuo* up to 500–600° C., and is probably a definite compound. Niobium iodides, when heated, gave products with the formula Nb_6I_{14} (? Nb_2I_5), whilst the formula V_2I_3 was calculated for the dark reddish-brown vanadium iodide obtained when the pentaiodide sublimes. Details are given for the various reactions together with the physical properties of some of the compounds. D. G. H.

Reactions of Resorcinol with Niobium and Tantalum. F. M. Shemjakin and V. A. Pilipenko. (*J. Ob. Chimie*, 1938, **8**, 824–828.)—It was stated by Platonov, Krivoshlikov and Marakaev (*J. Ob. Chimie*, 1936, 1815) that in ammoniacal solution resorcinol gives blue, bluish-green and greyish-green colours with niobium, tantalum and titanium respectively. As similar blue or green colours are also given by zinc, calcium and nickel, and as an ammoniacal or alkaline solution of resorcinol alone gradually gives a bluish-green colour which changes to yellowish-green, the reaction is obviously not specific for the above-mentioned metals. The authors have investigated the colour reactions of potassium niobium fluoride and potassium tantalum fluoride by the method of Gibbs's triangle. The tantalum compound was prepared by Treadwell and Hall's method, but with the use of Merck's perhydrol instead of nitric acid. The results for the 36 divisions of the triangles are illustrated for solutions of the fluorides in potassium hydroxide (results in ammoniacal solution in the presence of ammonium oxalate were negative). It was found that tantalum changed the colour of resorcinol from a greenish-yellow shade to yellow or yellowish-orange at a series of points; at a few points a flocculent yellow precipitate was formed. With niobium the same colour was usually obtained as with resorcinol alone, but in some regions the colour disappeared completely. These results also indicate that the reaction is not specific for niobium and tantalum. Similar colour reactions of cerium, other rare earths and their

related elements have been studied. Most of these yield a blue or bluish-green precipitate in ammoniacal solution. In a solution of potassium hydroxide the precipitates formed are of various colours. E. B. D.

Determination of Strontium in the Presence of Calcium. R. N. Shreve, C. H. Watkins and J. C. Browning. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 215.)—The solution containing strontium and calcium as nitrates is acidified with nitric acid and evaporated to dryness on a steam-bath. The mass is cooled, and 25 ml. of anhydrous acetone are added and allowed to remain in contact with the solid for one hour with occasional shaking. Calcium nitrate dissolves, and the residue of strontium nitrate is filtered off on a Gooch crucible, washed with acetone, dried and weighed. Tests on mixtures of 1 to 2 g. of strontium nitrate with 0·1 to 2 g. of calcium nitrate, showed that the percentage recovery of strontium nitrate was 99·7 to 99·9. Strontium and calcium, present as chlorides, may be conveniently converted into nitrates by precipitating them as carbonates with ammonium carbonate and dissolving the washed precipitate in nitric acid. S. G. C.

Colorimetric Determination of Chlorine with *p*-Aminodimethylaniline. D. H. Byers and M. G. Mellon. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 202–203.)—A spectrophotometric study of this method for the determination of residual chlorine in water has been carried out. A purple colour is produced, and the sensitiveness is 0·01 to 0·03 p.p.m. It is stated that the method seems to have no advantage over the more familiar *o*-tolidine method unless there is an individual preference for matching purple rather than yellow hues. S. G. C.

Improved Molybdenum Blue Reagents for Determination of Phosphorus and Arsenic. J. A. Schricker and P. R. Dawson. (*J. Assoc. Off. Agric. Chem.*, 1939, **22**, 167–179.)—In Zinzadze's method for the quantitative determination of minute concentrations of phosphorus and arsenic (*Z. Pflanzenern. Düng. u. Bodenk.*, 1930, **16** (A), 129; 1932, **23**, 447; Abst., ANALYST, 1932, **57**, 411; *Bull. Soc. chim.*, 1931, **49**, 872; *Ind. Eng. Chem., Anal. Ed.*, 1935, **7**, 227, 230; *Z. Unters. Lebensm.*, 1935, **70**, 308; Abst., ANALYST, 1936, **61**, 198) the conditions for minimum interference by silica and salts have been studied. For phosphorus they are: molybdic oxide concentration, 0·0018 *M*; sulphuric acid concentration, 0·36 *N*; and reduction concentration, 0·0004 *N*; for arsenic the same sulphuric acid and reduction concentrations are required, but the molybdic oxide concentration should be 0·0032 *M* (the term "reduction concentration" applies to the concentration of quinquevalent molybdenum oxide resulting from reduction of the sexavalent molybdenum oxide). To attain these conditions the concentrated reagent for phosphorus has molybdic oxide, sulphuric acid, and reduction concentrations of 0·18 *M*, 36 *N*, and 0·040 *N* respectively, whilst the molybdic oxide concentration in the arsenic reagent has the value 0·32 *M*; the reagents are therefore conc. sulphuric acid solutions of 0·02 *M* $Mo_{10}O_{29}$ for phosphorus and 0·02 *M* $Mo_{17}O_{50}$ for arsenic. Zinzadze's reagent is further modified by the substitution of quinaldine red for β-dinitrophenol as an indicator for preliminary adjustment of the *p*H of the test solution, so that the final *p*H remains well below 1·0

when the molybdenum blue reagent is added, and by the use of sulphurous acid instead of sodium bisulphite. The following methods are recommended.

METHOD A, PHOSPHORUS.—(*a*) *Molybdenum blue for phosphorus.*—Pure molybdenum trioxide is ignited in a porcelain dish at a dull red heat and below its m.p. in a muffle furnace for about 1 hour and then cooled. A quantity of 6·96 g. is boiled gently in a 500-ml. Kjeldahl flask with 250 ml. of conc. (36 *N*) sulphuric acid and a few glass beads until solution is complete (a slight cloudiness does not matter). The liquid is cooled to about 150° C. and 0·16 g. of pure powdered molybdenum metal is introduced carefully down the neck of the flask. The flask is rotated occasionally for 10 minutes, after which the metal should be completely dissolved; if there is any residue the liquid is heated again to 150° C. When solution is complete the liquid is cooled, and a convenient portion (5 or 10 ml.) is diluted with about 5 volumes of water and titrated with 0·1 *N* or 0·02 *N* potassium permanganate solution, the end-point being the appearance of a pink colour lasting for 1 minute. The reduction concentration of the solution, which should be about 0·04 *N*, is calculated from the result. If the reduction concentration is less than 0·036 *N*, a calculated quantity of molybdenum sufficient to bring the concentration up to about 0·04 *N* is added and the liquid is re-heated to 150° C. and then cooled, and the titre is checked again. The bright blue solution is kept in a glass-stoppered bottle. (*b*) *Standard phosphate.*—Aqueous monopotassium phosphate solutions containing 1, 10, 100, etc., γ of phosphorus per ml. are prepared. (*c*) *Determination of phosphorus.*—To an aliquot part of the phosphorus solution in a 50-ml. volumetric flask there is added 1 drop of 0·01 per cent. aqueous quinaldine red solution, followed by dilute solutions of sodium carbonate or sulphuric acid until the red colour just disappears. Water is added to about 25 ml. and then 5 ml. of a saturated solution of sulphur dioxide or of a solution obtained by dissolving 5·2 g. of pure sodium bisulphite in 100 ml. of *N* sulphuric acid (this solution must be prepared weekly and kept stoppered). The liquid is digested on the steam-bath for 30 minutes or for 20 minutes after the temperature has reached 95° C.; if the sample is comparatively free from arsenic and nitrate (not more than 50γ) this time may be shortened to 10 minutes. Half a ml. of the molybdenum blue reagent is run down the neck of the flask, to avoid spurting. The neck of the flask is rinsed down with two or three drops of water, the liquid is mixed, and digestion is continued for 30 minutes. The liquid is cooled, made up to 50 ml. and mixed, and the colour is read against a standard similarly prepared or, preferably, in a photo-electric colorimeter. It is desirable to read the colours within 4 hours, as there is a 2 to 5 per cent. fading during the first 24 hours, but this difference is detectable only by a photometer, so that the fading does not preclude the reading, by ordinary colorimetric methods, of colours left overnight. After 24 hours, if the flasks are stoppered, the colours remain quite stable for a week or more, even when exposed to light.

METHOD B, PHOSPHORUS.—(*a*) *Sulphomolybdic acid.*—A quantity of 7·2 g. of ignited pure molybdic oxide is dissolved in 250 ml. of conc. sulphuric acid as in method A. The reagent is cooled and stored in a glass-stoppered bottle. (*b*) *Determination of phosphorus.*—The method is the same as method A up to the end of the digestion with sulphurous acid or sodium bisulphite. Half a ml. of the

sulphomolybdic acid reagent and 1·0 ml. of metol solution (0·42 g. of methyl-paramidophenol sulphate and 6·3 g. of sodium sulphite in 100 ml.) are pipetted in with the same precautions as in A. The digestion is continued for 30 minutes on the steam-bath, and the liquid is cooled, made up to volume, mixed, and read as in A. In the absence of appreciable quantities of arsenic and nitrate the preliminary digestion may be shortened as in A. These reagents are affected by iron, arsenic and nitrate when the quantities of these are greater than 10 mg., 1 mg., and 1 mg., respectively. The sensitivities of the two methods for phosphorus are practically identical, but method B shows a somewhat more rapid development of colour.

METHOD C, ARSENIC.—(*a*) *Molybdenum blue for arsenic.*—The procedure is the same as for preparing the molybdenum blue reagent for phosphorus under A, but 12 g. of molybdic oxide is used per 250 ml. of sulphuric acid. (*b*) *Standard arsenate.*—Aqueous monopotassium arsenate solutions containing 1, 10, 100, etc., γ of arsenic per ml. are prepared. (*c*) *Determination of arsenic.*—The aliquot part must be free from nitrate, phosphorus and arsenic in the quinquevalent form (a previous separation by trichloride distillation into water followed by evaporation of the distillate with an excess of nitric acid to the complete elimination of hydrochloric acid and nitric acid is probably the most satisfactory: Deemer and Schricker, *J. Assoc. Off. Agric. Chem.*, 1933, **16**, 226). The residue is dissolved and washed with hot water into a 50-ml. flask, and the liquid is neutralised to quinaldine red, as directed under A and B, and made up to about 30 ml. Half a ml. of the molybdenum reagent is added, and the liquid is digested on the steam-bath for 30 minutes, cooled, made to volume, mixed and read as directed under A (see also Abstract, p. 432–434). E. M. P.

Microchemical

Applications of Spot Tests to Analysis of Medicinal Preparations. VI, VII, VIII, and IX. O. Frehden and K. Fürst. (*Mikrochem.*, 1938, **25**, 256–258; 1939, **26**, 36–38, 38–40, 40–43.)—VI. *Test for formic acid.*—The test, which depends on the detection of carbon dioxide liberated by the action of bromine ($HCOOH + Br_2 \rightarrow 2HBr + CO_2$), is made in Feigl's apparatus (F. Feigl, *Spot Tests*, English trans., 1937, p. 11, Fig. 16) for micro-distillation and collection of evolved gases. A few drops of the test solution are placed in the bulb of the apparatus and treated with bromine water, drop by drop, until the yellow colour persists. A few splinters of quartz are added to prevent spurting, a drop of a saturated solution of barium hydroxide is placed in the glass tube which serves as a stopper for the apparatus, and the bulb is heated over a micro-burner until its contents boil. The baryta turns cloudy when formic acid is present. For small amounts a blank test is advisable. The barium hydroxide is covered with a thin layer of liquid paraffin to prevent it coming into contact with the air. As little as 2·5γ of formic acid may be detected.

VII. *Test for polyhydroxy compounds.*—The formic acid test (see above) may be applied after oxidation of the polyvalent alcohols with periodate in sulphuric acid solution to formaldehyde and formic acid. A little of the sample is placed in Feigl's apparatus (see above) together with 2 drops of a saturated solution of sodium

periodate and 2 drops of *N* sulphuric acid, and warmed gently. The procedure described above, for formic acid, is then followed. The following substances were tested, the amounts in brackets being the smallest quantity to give a positive reaction in each instance:—glycerol (2·5γ), mannitol (3γ), dextrose (5γ), laevulose (5γ), arabinose (5γ), mannose (2·5γ), maltose (2·5γ), sucrose (7γ), lactose (6γ), dextrin (6γ), starch (20γ), and mucic acid (3γ). The test may be carried out in the presence of aldehydes, with the exception of formaldehyde.

VIII. *Test for aldehydes.*—The test depends on the fact that malachite green which has been decolorised with sodium sulphite regains its colour on the addition of aldehydes. The test may with advantage be carried out on a stable reagent paper, prepared as follows:—0·8 g. of malachite green is dissolved as the leuco-base by heating it in presence of 3 g. of sodium sulphite; a further 2 g. of sodium sulphite are then added and the mixture is filtered. On cooling, crystals of the leuco-base are precipitated from the filtrate, which is then only pale yellow. Strips of thin filter-paper are immersed in the yellow solution and dried in the air. The impregnated paper should be quite white without even a trace of green; when stored in a stoppered bottle it will keep for several weeks. When a drop of the neutral test solution is added to the reagent paper a green colour indicates aldehydes. The solution must be neutral, since both acids and alkalis change its colour. In a series of tests with 20 aldehydes amounts varying from 20 to 300γ were detected.

IX. *Test for free chlorine and substances containing chlorine.*—This test, due to Frehden and Chen-Hua Huang, depends on the interaction of chlorine with potassium bromide to liberate bromine, which is recognised by its action in converting fluorescein into eosin. Stable spot-paper for the reaction is prepared by immersing it in a solution of 0·1 g. of fluorescein in 100 ml. of dilute alkali containing 0·5 to 0·8 g. potassium bromide, and drying it in the air. When the vapour or solution to be tested is brought into contact with the pale yellow reagent paper a violet-red spot is formed if free chlorine is present. The test is not applicable in the presence of other free halogens, but other oxidising agents in general give no reaction, and the test is therefore preferable to one involving the use of starch-iodide paper. Chlorides can be detected after liberation of their chlorine by heating with sulphuric acid and manganese dioxide. The test can be used for the detection of free chlorine in washing soaps, bleaching agents, medicinal chloroform, drinking water, dyes, gases, etc. J. W. M.

Micro-test for Palladium. R. Schoental. (*Mikrochem.*, 1938, **24**, 20–21.)—Solutions of *p*-amino-acetophenone form a yellow complex salt with palladium salts in neutral or slightly acid solutions. The complex salt is insoluble in cold water, dilute acids, alcohol, ether, acetone or chloroform. Strong alkalis decompose both the reagent and the complex salt. The reaction is extremely selective and may be used for the separation of palladium from other cations, especially in the platinum group (Pt, Ir, Os, Ru, Rh), and also from gold and tellurium. Only cerium salts interfere and must be removed before carrying out the test. The reagent is used in a 1 per cent. aqueous solution containing 2 ml. of conc. hydrochloric acid. When the test solution is mixed with an equal volume of the reagent a cloudiness is perceptible with as little as 5γ of palladous chloride

per ml. When the test is made on a microscope slide and the precipitate is examined under the microscope 0·75γ of palladous chloride can be detected. The reaction may also be adapted to quantitative nephelometric analysis. J. W. M.

Micro-determination of Manganese in Biological Materials. A. C. Wiese and B. C. Johnson. (*J. Biol. Chem.*, 1939, **127**, 203–209.)—A method capable of determining 0·1 to 10γ of manganese has been based on oxidation of the manganese to permanganate with sodium bismuthate and addition of the permanganate solution to a strongly acid solution of benzidine in nitric acid. This causes the formation of a yellowish-green colour instead of the blue colour obtained in dilute acetic acid solution; the former colour is much more stable. A sample containing 1 to 10γ of manganese is weighed and ashed in a well-extracted porcelain dish at cherry-red heat. The ash is digested with a boiling mixture of 3 to 5 ml. of conc. nitric acid and 10 ml. of water, and the solution is transferred to a beaker and evaporated to dryness. The residue is digested with 3 to 5 ml. of conc. nitric acid and 10 ml. of water and the solution is evaporated. This process is repeated a third time to ensure complete removal of chlorides, which cannot be removed by precipitation with silver nitrate solution, as excess of this interferes in the later stages of the determination. The final residue is dissolved in 3 ml. of conc. nitric acid and 10 ml. of water, and the solution is boiled for 2 to 3 minutes with 0·2 g. of sodium bismuthate to remove oxides of nitrogen. After being cooled to below 30° C. the solution is mixed with 0·3 to 0·5 g. of sodium bismuthate and left for a few minutes. The excess of sodium bismuthate is filtered off in a Gooch crucible, and the filtrate is collected in a colorimeter tube containing 3 ml. of redistilled water and 2 drops of a 1 per cent. solution of benzidine in 5 per cent. acetic acid. (The benzidine used was specially purified by crystallisation from 70 per cent. alcohol, decolorisation with charcoal and recrystallisation, followed by a second recrystallisation from benzene.) The solution is made up to 25 ml. and thoroughly mixed. The yellowish-green colour that develops is measured after 5 minutes in an Evelyn photo-electric colorimeter with a 420$m\mu$ filter. The instrument is calibrated by means of solutions containing known amounts of manganese and treated in the same manner. A Dubosq colorimeter can be used instead of the photo-electric colorimeter, but the procedure is not so sensitive or so rapid. Amounts of manganese between 0·1 and 1·0γ were determined with an error of 20 to 30 per cent., but with amounts between 2 and 15γ, the error was generally only about 2 per cent. When manganese was added to various materials recoveries ranging from 92 to 106 per cent. were obtained. F. A. R.

Physical Methods, Apparatus, etc.

Determination of Water in Acetone by means of the Infra-red Absorption-Spectrum. R. Gaspart and L. Gillo. (*Bull. Soc. Chim. Belge*, 1939, **47**, 933–939.)—The infra-red absorption-spectrum of mixtures of acetone and small amounts of water has a characteristic double band consisting of an intense band of wave-number 3550 cm.$^{-1}$ corresponding with a state of molecular association

between water and acetone, and a less intense band of wave-number 3580 cm.$^{-1}$* Methods based on the measurement of these bands have already been described and applied to the determination of 250 parts per 100,000 of water in acetone (*cf.* Errera and others, *Physica*, 1937, **4**, 1097; 1938, **5**, 116; *cf.* also *Trans. Faraday Soc.*, 1938, **34**, 729), and the present authors have now extended them to acetone containing even smaller concentrations of water. The acetone was distilled in a Swietoslawski ebullioscope provided with 2 Beckmann thermometers showing the temperature of the boiling liquid and of the condensing vapour. By means of a calibration-curve it was possible to determine the concentration of any mixture from the difference between the two temperatures. The outlet of the apparatus was connected directly with the observation cell, the exit of which was connected through a condenser with a receiver, all outlets to the air being protected by tubes containing phosphorus pentoxide. At the beginning of the distillation the cell was "rinsed," and all traces of moisture were removed from the air within it by maintaining it at a temperature slightly above the b.p. of acetone; the inlet and outlet tubes of the cell were finally sealed in a flame. It was thus possible to fill the cell with pure acetone or, by the use of constant-boiling mixtures, with acetone containing known quantities of water. Curves are shown relating the wave-number of the light used (3800 to 3400 cm.$^{-1}$) with the percentage of light transmitted by a layer of 1 ml. of the sample (1 mm. thick), as compared with carbon tetrachloride; corrections were applied for reflection effects produced at the parallel faces of the cells. A comparison of the curves for pure acetone and for acetone containing 1, 2·5 and 5 parts of water per 100,000 shows that quantities of water of the order of 1 part per 100,000 may be detected from the marked depression in the transmission ratio at a wave-number of 3618 cm.$^{-1}$, corresponding with the more intense of the bands due to water. It is then shown how the actual concentrations of water may be determined from the formulae, $T = e^{-kd}$ and $T = e^{-xcd}$, where T is the measured transmission, k the coefficient of extinction, x the molecular coefficient of extinction, c the concentration of water (in g.-mol. per litre), and d the thickness of the cell in cm. The concentrations of water found for the three solutions were, respectively, 1, 4 and 9·5 ($\pm$ 1) parts per 100,000 for the ebulliometric method; 0·7, 2·2 and 5 parts per 100,000 for the spectroscopic method, the possible errors for the latter being 100, 25 and 10 per cent., respectively. The values of x obtained for the three solutions (2090, 1850 and 1260, respectively) decrease as the concentration increases, so that the Beer-Lambert law is not followed completely, and therefore for exact quantitative work it is necessary to work from a calibration curve based on experimental values obtained with solutions of known concentrations and showing the relationship between k and c. J. G.

* The frequency, *i.e.* the number of vibrations per second, is found by dividing the velocity of light by the wave-length λ, but spectroscopists have found it more convenient to use the simple reciprocal of the wave-length, *i.e.* $^1/\lambda$ instead of $^c/\lambda$, λ being expressed in cm. This reciprocal gives the number of waves per cm. length and is known as the wave-number. For example, a wave-number of 3618 cm.$^{-1}$ corresponds with a wave-length of $\frac{1}{3618}$ cm. = 2·764 $\times$ 10^{-4} cm. = 2·764μ.—EDITOR.

Errata—*February Issue*

P. 133, l. 6 from bottom, *for* "2 litres of water are added" *read* "made up to 2 litres."

P. 134, l. 1, *for* "in strong daylight" *read* "strong daylight being avoided."

P. 135, l. 6, *for* "$0{\cdot}2G^2$" *read* "$0{\cdot}02G^2$."

P. 134, l. 18, the prescription of 50 ml. is unnecessary.

Reviews

SPECTROCHEMICAL ANALYSIS IN 1938. A companion book to "Spectrochemical Abstracts 1933–38." By F. TWYMAN, F.R.S. Pp. 68. London: Adam Hilger, Ltd. Price 4s. 4d.

As the title indicates, this monograph adds to the information given in the earlier publications. It incorporates two reports published in the Annual Report of the Chemical Society for 1937 and in the *Journal of the Society of Chemical Industry*, 1938. Both were well worth reprinting, the first because it deals generally with various methods of exciting spectrum lines and with their measurement; the second with the theory and practice of microphotometry as applied to the quantitative determination of the constituents of alloys when present in small amounts. The latter report treats in detail of precautions necessary for the examination of alloys and is illustrated by reference to the quantitative analysis of aluminium alloys. A short chapter is devoted to discrepancies between chemical and spectrochemical analysis, and reasons are advanced for attributing the discrepancies to the heterogeneity of the alloy. If this be accepted, it follows that in important cases chemical analysis should be employed to obtain a measure of the overall proportion of a constituent or impurity, and spectrochemical analysis to ascertain the variation in this constituent. When the proportion of the constituent is small in amount, say less than 0·3 per cent. or thereabouts, the spectrochemical method is accurate enough to show when heterogeneity is present. A few pages are devoted to sparking circuits, and a very good case is made out for considering that the methods developed by Twyman and his co-workers are as accurate and convenient as any of the more elaborate methods proposed abroad.

Mr. A. C. Candler summarises usefully the elements of the theory of atomic spectra. The reviewer's only comment on this summary is that it is so condensed that the beginner will have to consult more complete accounts if he desires to understand the subject fully. One such account is furnished in Candler's two volumes on "Atomic Spectra," where the subject is dealt with in much more detail.

An important aim of the monograph is to give the best technique for routine analysis of metals or alloys. Taken in conjunction with earlier monographs issued by the present publishers, this claim can be accepted as established.

It is gratifying to note that in the last few years an impressive number of British firms, individuals and institutions have turned to spectrochemical methods of analysis, and it is fortunate that the wide experience of the staff of Messrs. Hilger is available in this field.

J. J. Fox

HANDBOOK OF CHEMICAL MICROSCOPY. Vol. I. By E. M. CHAMOT, B.S., Ph.D., and C. W. MASON, A.B., Ph.D. Second Edition. Pp. xvi + 478, with 165 figures. London: Chapman & Hall, Ltd. 1938. Price 22s. 6d. net.

There is perhaps no other physical instrument adaptable to such a wide range of uses as the microscope, and the contents of this volume serve as a detailed guide to its manifold applications.

At the present time the terms micro-chemistry and chemical microscopy are not infrequently confused, but whilst the former should be applied to the detection and estimation of extremely small amounts of substances for which magnification is rarely necessary, the latter refers to the use of the microscope in the observation of the results of chemical reactions, and in qualitative and quantitative physical and physico-chemical determinations on a wide range of pure and industrial materials. These procedures have many advantages over the usual methods of examination, notably in the small size of the sample required, the avoidance in many instances of the destruction and loss of the material and the great saving of time ensured by eliminating tedious chemical analyses.

The arrangement of the subject-matter in the present volume is identical with that of the previous edition reviewed in THE ANALYST (1930, **55**, 470) and has copious foot-note references throughout the text.

The field of chemical microscopy is rapidly extending and this has necessitated the insertion of much new matter of considerable value. This includes the application of the Christiansen effect to colour filters in which the transmitted light is dependent upon temperature, "polaroid" filters, the centrifuge microscope, the "electron microscope" in which extremely high magnifications are obtained by the use of rays having a wave-length of 0·05 A.U. the Jelley grating microspectrograph, and the newer types of low-voltage incandescent electric lamps. For the benefit of the student descriptions are given of over one hundred experiments relating to the use of polarising apparatus, determination of the optical characteristics of various isotropic and anisotropic substances, the preparation of crystals and the determination of the refractive indices of solids and liquids. These in conjunction with the synopsis, in the appendix, of the laboratory practice adopted at Cornell University, form an excellent basis for training in the application of microscopic methods. So much of the chemist's ordinary physical work is now becoming mechanised that there is a great tendency towards a development of mental stasis in its application, but in the practice of chemical microscopy the mind of the observer must be continually on the alert to interpret correctly the phenomena observed.

The authors have covered a very wide field in their book, although Barger's molecular weight method and the vapour density method of Blackman appear to have been omitted, but these may perhaps be given in Vol. II, to be published later. Very brief references only are made to mineralogy, metallography and the biological sciences, since these are adequately dealt with in other works, but the general methods described in this volume are in many instances applicable to these branches of science.

The text is thoroughly up to date, including references to apparatus and methods described up to July, 1938, and it has been produced with the care and

precision displayed in the earlier edition. A few very minor typographical errors are evident, and the transatlantic source of the volume is indicated by the presence of such verbs as "diaphragmed" (p. 45), "paralleling" (p. 77) and "diagrammed" (p. 126)—words that would make the flesh of our etymologists creep. A high degree of accuracy is evident in the text, references and index, but it may be suggested that the index would be of greater value had more cross-indexing been introduced.

This production maintains the lucidity and reliability of its predecessors, and may be thoroughly recommended, not only to the student, but to all engaged in the practice of pure or applied chemistry, for in addition to its instructional value it forms a comprehensive guide to most of the international literature of the subject.

T. J. WARD

RECENT ADVANCES IN CHEMOTHERAPY. By G. M. FINDLAY, C.B.E., M.D., D.Sc. 2nd Edition. Pp. x + 523. London: J. & A. Churchill, Ltd. 1938. Price 21s.

As a result of the recent discovery of the sulphonamide drugs, interest in chemotherapy has been greatly stimulated and the search for new chemotherapeutic substances is being more actively pursued throughout the world. The timely appearance of the second edition of this book will therefore be much appreciated, presenting as it does an up-to-date survey of the knowledge and progress in this field. Indeed, the title scarcely does justice to the wide scope of the treatment, for the earlier work is as carefully summarised as the more recent discoveries.

In the course of revision the book has been practically re-written in order to make room for accounts of important advances and to augment the earlier work with further clinical results. Nevertheless, the essentials of the original form are retained, a chapter being allocated to each infection or group of infections, ranging from those due to helminths to diseases resulting from ultra-microscopic viruses.

Many new chemotherapeutic substances are described and, where available, full information is given regarding their clinical effectiveness, toxicity, pharmacology and use in treatment and prophylaxis.

Among the anti-malarials Plasmoquin and Atebrin represent a considerable advance, although quinine still ranks first on account of its effectiveness, low toxicity and the widespread familiarity with its use. The search for more active and less toxic substances is still proceeding, and the results of investigations of numerous homologues of Plasmoquin are given.

In the chapter on trypanosomiasis new organic arsenicals are described, notably the aryl thioarsinites. In addition, there are the interesting polymethylene amidines and guanidines whose constitution is entirely different from that of any other known trypanocides. Undecamethylene diamidine, the most active and least toxic member of the series, is also reported as having a temporary action on certain types of malaria.

The most outstanding developments recorded are, of course, in connection with the sulphonamide drugs. The discovery of these substances is described by the author in his preface as equal to, if not greater than, the introduction of

Arsphenamine by Ehrlich. This is surely no exaggeration, as an examination of the long chapter on acute bacterial infections will prove. Although it is not possible here to give more than the briefest summary of this section, it may be said that it represents a condensed account of all important developments in the subject, from the discovery of Prontosil to the appearance of M. and B. 693, which is proving so useful in the treatment of pneumococcal infections.

Mention must also be made of the diphenyl sulphones which have been shown to possess, for certain experimental diseases, a therapeutic effect comparable with that of sulphanilamide.

Another advance, which has been rather overshadowed by the success of the sulphonamide drugs, is the use of mandelic acid in the treatment of bacillary infections of the urinary tract. Although, in some instances, it has been shown to be inferior to sulphanilamide, mandelic acid is far less toxic and is highly effective in certain streptococcal infections for which sulphanilamide is practically useless.

The chapter on virus infections is interesting because there are indications that a successful chemotherapy is becoming possible. The stimulus is again provided by sulphanilamide which has been shown to be effective in the case of at least one disease of this origin.

The only chapter that has been omitted from this edition is the short one on cancer, and in view of all the successes in the other fields one's hopes are raised that even this disease will eventually yield to chemotherapy.

Whilst this book may be of greatest value to the experimental pathologist and clinician, yet there is much to interest the organic chemist who may be called upon to produce new substances for chemotherapeutic investigation. Although no general relation between structure and therapeutic effect is discernible, variations in the molecule of certain drugs are seen to follow rough rules as regards the effect upon activity and toxicity. These generalisations are recorded and will be of considerable value in connection with the synthesis of derivatives and homologues of known active substances.

In conclusion, attention must be drawn to the excellent bibliography appearing at the end of each section, to which reference is made in the text.

G. E. H. SKRIMSHIRE

FUNDAMENTAL PRINCIPLES OF BACTERIOLOGY, WITH LABORATORY EXERCISES. By A. J. SALLE, B.S., M.S., Ph.D. First Edition. Pp. 679. London: McGraw-Hill Publishing Company, Ltd. 1939. Price 24s. net.

This book deals with fundamental principles and facts concerning the science of bacteriology. It is primarily intended for beginners, and the author has endeavoured to give explanations of all phenomena described, but it is not merely elementary; it is intended to provide the student with a solid foundation upon which to build for more specialised work and will be found to contain a great deal of detail. Thus in the chapter on Nutrition we find a good account of the requirements of different groups of bacteria:—the groups that require typtophane and the groups that do not; the groups that require vitamins and the groups that can synthesise their own; of the V and X factors required by *Haemophilus influenzae*. The essential organic and inorganic chemistry involved in staining, disinfection,

enzymes, respiration, industrial fermentations, nutrition and so forth are included. The biological stains are classified, graphic formulae are given and chromophores are indicated. Thus the book is written as a combined text-book and laboratory manual, the experimental portion being woven into the body of the text under the appropriate chapters.

The headings of the chapters, which are as follows, convey a fair idea of the scope of the work:—Introduction, General Laboratory Instructions, The Nutrition of Bacteria, The Microscope, Biological Stains, The Morphology of Bacteria, The Yeasts, The Moulds, Pure Culture Technique, Influence of Environment upon Bacteria, Disinfection and Disinfectants, Enzymes of Bacteria, The Respiration of Bacteria, Protein Decomposition, Industrial Fermentation, The Differentiation and Classification of Bacteria, The Bacteriology of Water, The Bacteriology of Soil, The Bacteriology of Air, The Bacteriology of Milk and Milk Products, The Bacteriology of Foods, Bacterial Diseases of Plants, Bacterial Dissociation, Infection and Immunity, Specific Infections, History of Bacteriology, Preparation of Solutions and Stains, and Preparation of Culture Media. These subjects are fully dealt with and anyone who carefully studies the book and carries out a wise selection of the laboratory procedures described (of which there are no less than 111) will most certainly acquire a very ample and sound foundation from which to specialise in any of the branches of bacteriology.

The book is well written and arranged, and has a good index; it is well printed on excellent paper and attractively bound. From every point of view it is to be strongly recommended. D. R. WOOD

UNTERSUCHUNGSMETHODEN FÜR ARZNEISPEZIALITÄTEN. Fédération Internationale Pharmaceutique. Leyden. Second Edition. Pp. 148. Amsterdam: Dekker & Nordemann. 1938. Price Fl. 3·50.

There is no doubt that medicaments are ideal substances for administration in tablet form, and the public has no reason to complain that manufacturers fail to cater for its wants. Some will maintain that the glut of such articles has the disadvantage of encouraging self-medication; but it is certain that the convenient form of these preparations ensures their retention on the market, and an increase in number is to be expected in the future. The examination of such products is essential both for manufacturing control and for the protection of the purchaser; if well-tried methods can be standardised, advantage accrues to both maker and user. Such methods are usually available for pharmacopoeial or other official preparations; but the vast majority of tablets and pastilles are unofficial, and analysts are grateful when methods of standing are available for their examination.

The present volume is the second edition of methods of analysis for unofficial preparations drawn together by a Commission appointed by the Fédération Internationale Pharmaceutique. The Commission comprises representatives from Holland, Denmark, France, Sweden, Austria, Switzerland and Germany. The contents of the book are divided as follows:—10 pages to general physical methods, 7 to general chemical methods, 2 to the wet oxidation process for decomposition, 44 to quantitative determination of elements and certain compounds, 56 to the examination of tablets and pastilles (including a table of 88 substances with notes

on their quantitative analysis), 3 to the determination of rarer metals, 8 to indicators and reagents, 8 to three tables, and 10 to preface, contents and index.

The methods appear to be wisely chosen, and sufficient practical details for standard technique are given. Inconsistency is shown in the references to original work. While some methods are correctly ascribed, some have no reference and others are attributed to authors who can by no means be regarded as originators. For example, the general method for determining alkaloids—extraction with chloroform from alkaline solution, evaporation and titration—is ascribed to papers in 1931 and 1932. Of the three tables given at the end of the book, two are acknowledged as taken from the United States and the Dutch Pharmacopoeias respectively, whilst there is no mention, other than in the Index, that the third is copied from the British Pharmacopoeia.

While dealing almost exclusively with analytical methods, standards are laid down for firmness, disintegrability and weight of tablets, and the quantity of active substance which should be present. The requirement for firmness is that tablets and pastilles, while kept in original containers, must not crack, nor the corners be appreciably broken. Three standards of disintegrability apply to tablets (except those which are required to dissolve slowly in the mouth), *viz.*,

(1) Tablets without coating must, after 10 minutes in water at body temperature, be dissolved, disintegrated or so far softened that they crumble with the slightest pressure.

(2) Coated tablets placed in water at body temperature must be so softened within 2 hours that both coating and content crumble with the slightest pressure.

(3) Tablets for absorption in the intestines must not be disintegrated within 2 hours at body temperature in water or in 0·1 *N* acid. In alkaline medium they must be sufficiently softened in 1 hour to crumble with the slightest pressure.

The standard for weight to ensure uniformity is as follows:

Weight of individual tablets	Greatest variation in 90 per cent. of the tablets
Below 0·25 g.	12 per cent.
0·25 to 0·50 g.	10 ,, ,,
Over 0·50 g.	6 ,, ,,

The remaining 10 per cent. of the tablets must not vary by more than double these amounts. The quantity of active substance present must not vary by more than 10 per cent. from the declared quantity (tablets within the above limits of weight being used).

These requirements and standards appear reasonable and should meet the practical considerations of manufacturers as well as the needs of the purchaser.

A few errors have been noted, but the only one of possible significance is in Table II where, owing to the figures spreading over several pages, the line which should be *over* certain digits on the top of the page, appears *under* digits at the bottom of the previous page.

The book is well printed and has an excellent index. It should prove of great value to those whose work requires the analysis of certain unofficial but not uncommon tablets.

J. R. NICHOLLS

ELEMENTARY ANALYTICAL CHEMISTRY, QUALITATIVE AND QUANTITATIVE. By C. G. LYONS and F. N. APPLEYARD. 13th Edition. Pp. 282. London: J. & A. Churchill, Ltd. 1938. Price 7s. 6d. net.

A book designed to fill the needs of a student working for a special examination can only be judged within the limits of that object. This one, based on the text of Clowes and Coleman, is intended to supply the descriptive chemistry and practical instruction required up to and including a pass degree in science, the preliminary scientific examination of the Pharmaceutical Society and the preliminary medical and dental examinations; in this object it should succeed admirably and concisely. It goes straight to the task of supplying, within the syllabus of the several examinations for which it is intended, the descriptive chemistry required, and stops at that; no space is devoted to the standing orders of any particular laboratory, to teaching the teacher, or to doing his work for him by lists of test questions.

If the original version and repeated revisions and editions are included, the book is now in its 27th thousand, but as it has been re-set, and the originators' names removed from the title page, it is obviously intended to be regarded as a new book, and as such may be appraised afresh.

As will be seen from some of the remarks above, it falls into the class of text-book that is more suited to those who "take chemistry" as part of the course for other professions than to those for whom it is the serious business of life; its chemistry is of that idealised type in which all ions are invariably present in sufficient quantity to be readily detected, and the limitations imposed by solubility product and co-precipitation are non-existent. It contains, however, a short section of nine pages on physico-chemical theory, with an indication where more may be found; just sufficient to remove from a student's mind any mistaken impression that chemistry is exclusively a descriptive science.

The somewhat old-fashioned appearance of the previous edition has been removed by a new set of illustrations; these are drawn in a surrealist style that shows no line to mark the rim of glass vessels, so that a beaker looks like a flat-bottomed letter "U," and a funnel like a hollow-stemmed "Y." Such unconventional diagrams will be considered by many a poor exchange for the artistic drawings of former editions. Take, for example, that of the stiff-cuffed wrists of the immaculate but invisible chemist of a bygone age, who has demonstrated so successfully and to so many students the proper method of pouring liquid from one vessel to another.

The task of bringing a time-honoured text-book into line with modern ideas, while keeping within the limits of a prescribed curriculum, is one not lightly to be undertaken. The present authors are to be congratulated on their production, which may be expected to meet for many years to come the needs of those for whom it is intended.

Reviews of the 11th and 12th editions appeared in THE ANALYST, 1931, **56**, 137, and 1935, **60**, 277.

F. L. OKELL

THE PLANT ALKALOIDS. By T. A. HENRY, D.Sc. 3rd Ed. Pp. viii + 689. London: J. & A. Churchill, Ltd. 1939. Price 42s.

The prophecy that alkaloidal chemistry will still be the playground of the organic chemist a century hence, is probably a safe one. At the present rate of development this can be said of few other branches of organic chemistry. There are various factors which contribute to this situation, the most important of which is the lack of a system of mobilising the supply of raw plant materials from all quarters of the globe. The Amazonian forest, for instance, is full of alkaloid-bearing plants and trees, but nothing less than a costly expedition, equipped with expert botanists, can at present make any of these available in an authoritative form to science. The low yield of some alkaloids is another limiting factor, contents of 0·01 per cent. being not uncommon. This may be met in some cases by cultivation, for the colouring matters and alkaloids of many annuals and biennials have been made available to the chemist by growing the appropriate plant on a large scale on farms. The cultivation of tropical climbers, however, presents a much more formidable problem.

A further justification for the introductory statement may be seen in the fact that of 137 different alkaloids taken at random, probably about 10 per cent. are alkaloids of known constitution. In the book under review there are, for example, 31 alkaloids containing 15 carbon atoms, 33 containing 16 carbon atoms, 26 containing 17 carbon atoms and 47 containing 17 carbon atoms. As the number of carbon atoms in an alkaloid increases, so does, as a rule, the difficulty of solving the problem of its chemical constitution. Greater progress could be made in many cases with a more ample supply of material, but a few alkaloids with constitutions as difficult to disentangle as that of brucine or strychnine, whose structures are on the verge of solution after 40 years of intensive effort, may considerably retard the march of progress by their complexity.

This, the third edition of Henry's *Plant Alkaloids*, is the golden key opening the gate to this entrancing field, and, indeed, a source of inspiration for new advances in medicinal chemistry. With the progress in physiology and pharmacology, alkaloids which have hitherto been laboratory curiosities are daily entering into the service of medicine, and will no doubt continue to do so. The book has been entirely re-written, and the author's decision to omit discussion of the purine bases seems to be fully justified. In view of the expansion of the subject, it is hardly likely that a better book covering the whole alkaloidal field will ever be written by one individual. In the present production the balance between historical matter, experimentally ascertained chemical facts and pharmacology is well nigh perfect. The reader needs no reminder that several of the chapters owe their authoritative character to the experimental work of the author and his colleagues. The book is not unduly expensive, and the reproductions of the formulae are excellent. The volume is destined to remain the standard of reference for many years to come, not only to the university professor in search of problems for his students, but to the fine chemical manufacturer and all those whose activities impinge on the rapidly extending field of medicinal chemistry.

HAROLD KING